



Prevalence of AmpC β -Lactamase and Regulatory Genes (LysR, QseB, and QseC) in Multidrug-Resistant *Klebsiella pneumoniae* Clinical Isolates from Al-Hillah, Iraq

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Abstract: *Klebsiella pneumoniae* is a critical opportunistic pathogen classified by the World Health Organization among priority drug-resistant organisms, owing to its capacity to acquire multidrug resistance (MDR) and express diverse virulence mechanisms. Understanding the molecular basis of resistance and the prevalence of regulatory genes in clinical isolates is essential for effective infection control. A total of 200 clinical samples (sputum and urine) were collected from hospitalized patients at two teaching hospitals in Al-Hillah, Iraq. Isolates were identified using the VITEK 2 Compact system, and antibiotic susceptibility testing was performed. Genomic DNA was extracted by the boiling method, and conventional PCR was used to detect five target genes: 16S rRNA, *ampC*, *lysR*, *qseB*, and *qseC*. Of 100 culture-positive samples, twenty (20%) were confirmed as *K. pneumoniae* with 99% probability. High resistance rates were recorded against cephalosporins (100%) and ceftazidime/ceftriaxone (90%), while amikacin showed the highest susceptibility (85%). The *ampC* gene was detected in 40% of isolates. All isolates (100%) carried 16S rRNA, *lysR*, and *qseB* genes, while *qseC* was present in 85%. The widespread resistance—even in isolates lacking the *ampC* gene—suggests the possible involvement of additional concurrent mechanisms, such as ESBLs, efflux pumps, and porin loss, although these were not directly tested in the present study. The universal prevalence of regulatory genes *lysR* and *qseB* underscores their role in bacterial adaptation and virulence. Combined molecular and phenotypic approaches are essential for comprehensive characterization of MDR *K. pneumoniae* in clinical settings.

Keywords: *Klebsiella pneumoniae*; AmpC β -lactamase; multidrug resistance; QseB; QseC

1. Introduction

Klebsiella pneumoniae is considered one of the most important opportunistic pathogens associated with hospital-acquired infections, playing a significant role in respiratory, urinary tract, and bacteremic infections, particularly among hospitalized or immunocompromised patients. The World Health Organization has classified it as a priority pathogen due to its remarkable ability to acquire multidrug resistance (MDR), making the treatment of infections caused by this bacterium an increasing clinical challenge [1]. Antimicrobial-resistant Gram-negative bacteria pose significant public health challenges in both clinical and environmental settings, as evidenced by surveillance studies from diverse regions [2]. The infectious disease burden in

Iraq encompasses multiple bacterial and viral pathogens [3], and resistant organisms have also been documented in regional water supply systems [4]. This widespread distribution can be attributed to a combination of virulence factors, environmental adaptability, and complex genetic systems that enhance its survival and dissemination in clinical settings. This bacterium is characterized by its ability to develop broad-spectrum resistance to antibiotics, particularly β -lactam antibiotics, through the production of various β -lactamase enzymes, most notably AmpC. AmpC enzymes play a major role in hydrolyzing cephalosporins and penicillins, and studies have shown that these enzymes may be either chromosomally encoded or plasmid-mediated, facilitating their spread among bacterial strains. Roberts et al. [5] demonstrated that *bla*CMY-2 is among the most prevalent AmpC genes in *K. pneumoniae* isolates, while inducible genes such as *bla*DHA-1 may remain undetected in routine laboratory tests, thereby increasing the clinical risk associated with these mechanisms. Furthermore, Rao et al. [6] reported that plasmids carrying AmpC genes have a high capacity for horizontal gene transfer among bacterial species, thereby contributing significantly to the dissemination of resistance. Mobile genetic elements, including integrons, further accelerate the horizontal transfer of resistance determinants among clinical bacterial populations [7].

In addition, the regulation of gene expression associated with these resistance mechanisms is not solely dependent on the presence of resistance genes. Rather, it is also influenced by complex regulatory systems, such as the LysR family, which is one of the largest families of transcriptional regulators in bacteria. This family plays a crucial role in regulating stress responses, virulence, and antibiotic resistance. Hennequin and Forestier [8] reported that the OxyR regulator, a member of the LysR family, contributes to biofilm formation and mucosal colonization, thereby enhancing bacterial persistence within the host. Moreover, Zhang et al. [9] demonstrated that this system confers oxidative stress resistance and increased systemic virulence, highlighting its importance in infection progression. The co-occurrence of these regulatory genes alongside resistance determinants, such as *ampC*, in clinical *K. pneumoniae* isolates from Iraq has not been previously characterized, representing a key novelty of the present study. The specific genes *lysR*, *qseB*, and *qseC* were selected because they represent distinct regulatory layers—transcriptional regulation and two-component signal transduction—that are mechanistically linked to virulence and environmental adaptation in clinical Enterobacteriaceae. Antibiotic resistance has been characterized across diverse bacterial species in Iraqi clinical settings, including macrolide-lincosamide-streptogramin B resistance profiles in *Staphylococcus* clinical isolates [10], reinforcing the regional significance of multidrug resistance monitoring. Another important regulatory system is the QseB/QseC two-component system, which senses environmental signals and regulates virulence-related gene expression. This system links bacterial signaling with host-derived signals such as adrenaline and noradrenaline, enabling the bacteria to adapt to the host environment. Fernandez-Ciruelos et al. [11] showed that this system interacts with other regulatory networks such as PmrAB, influencing colistin resistance and bacterial motility. Additionally, Khajanchi et al. [12] reported that the QseBC system can act as a negative regulator of biofilm formation in certain species, reflecting the complexity of its regulatory role.

The 16S rRNA gene is one of the most important tools used in molecular bacterial characterization, widely employed for bacterial identification and phylogenetic analysis. Because this gene is highly conserved, it serves as a reliable marker for accurate species identification even when phenotypic characteristics are ambiguous. Legese et al. [13] demonstrated that sequencing techniques, including 16S rRNA, play a significant role in understanding molecular resistance patterns and their dissemination. Similarly, Fu et al. [14] highlighted the importance of this gene in tracking transmission between animal and human sources, reflecting the broader epidemiological implications of this pathogen. The interaction between genetic resistance mechanisms, such as AmpC, and regulatory systems, such as LysR and QseBC, forms a complex network that enables the bacterium to adapt to environmental pressures and antimicrobial agents. Recent studies have shown that these interactions influence not only resistance but also virulence regulation and biofilm formation, thereby increasing the difficulty of controlling this pathogen in clinical settings. Based on the above, this study aimed to perform molecular characterization of the *ampC* gene and regulatory systems *lysR*, *qseB*, and *qseC*, in addition to confirming bacterial identity using the 16S rRNA gene in MDR *Klebsiella pneumoniae* isolates from clinical settings in Iraq, to understand the relationship between resistance

mechanisms and regulatory factors that contribute to increased virulence and environmental adaptability of this bacterium.

2. Materials and Methods

2.1 Collection of Clinical Samples

Clinical samples were collected from hospitalized patients at two teaching hospitals in Al-Hillah city, Iraq: Marjan Teaching Hospital and Al-Hillah Teaching Hospital. The Institutional Review Board of the University of Al-Qadisiyah approved this study. Informed consent was obtained from all participating patients before sample collection. Sample collection and laboratory procedures were conducted from 19 November 2024 to 31 December 2024. A total of 200 clinical specimens were obtained from different clinical cases, including sputum and urine samples.

2.2 Isolation and Cultivation of Bacteria

The collected specimens were cultured on MacConkey agar for primary isolation of bacterial pathogens. Inoculated plates were incubated at 37°C for 24 h. After incubation, bacterial colonies were examined for morphological characteristics, including colony size, shape, color, lactose fermentation, and mucoid appearance. Suspected colonies of *Klebsiella pneumoniae* were selected for further identification.

2.3 Identification of Bacterial Isolates

Selected isolates were identified using the VITEK 2 Compact system (bioMérieux, France) according to the manufacturer's instructions. Identification results confirmed that the isolates were *K. pneumoniae* with high confidence.

2.4 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the VITEK 2 Compact system (bioMérieux, France). The AST-N291 Gram-negative susceptibility card was used. Minimum inhibitory concentrations (MICs) were determined automatically, and results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to the Clinical and Laboratory Standards Institute (CLSI) breakpoint guidelines (CLSI M100, 34th edition, 2024). Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, according to the standardized international definitions proposed by Hennequin et al. [8].

2.5 DNA Extraction

Genomic DNA was extracted from overnight cultures of *K. pneumoniae* using the boiling method. Briefly, a loopful of bacterial colonies was suspended in sterile distilled water and heated at 95°C for 10 min. The suspension was then centrifuged at 10,000 rpm for 5 min, and the supernatant containing DNA was collected and stored at -20°C until further use. All molecular biology reagents (e.g., agarose, loading dye, DNA ladder) were supplied by Geneaid (Taiwan). It is acknowledged that the boiling extraction method may yield lower-quality DNA than commercial spin-column kits, particularly for mucoid *K. pneumoniae* strains; however, successful amplification of all target genes confirmed the suitability of the DNA for PCR analysis.

2.6 Primer Design

The primers used in this study are listed in Table 1. Primers for amplification of the 16S rRNA gene were adopted from Liu et al. [15], universal primers widely used for bacterial 16S rRNA amplification and compatible with Enterobacteriaceae identification. Primers for amplification of *ampC*, *lysR*, *qseB*, and *qseC* genes were designed using Primer3Plus software based on published gene sequences and were synthesized commercially. The *ampC* primers were designed to target the blaCMY-type AmpC family, based on the reference sequence deposited in GenBank (Accession No. [INSERT ACCESSION NUMBER]). Authors confirm that in silico BLAST analysis was performed to verify primer specificity against *K. pneumoniae* sequences and to rule out cross-reactivity with other Enterobacteriaceae. Reference GenBank accession numbers used for primer design of *lysR*, *qseB*, and *qseC* are [INSERT ACCESSION NUMBERS]. All newly designed primers were validated against a known PCR-positive control before clinical sample analysis.

Table 1. Primers are used for PCR amplification of target genes.

Gene	Primer	Sequence (5'→3')	Annealing Temp (°C)	Product Size (bp)
16S rRNA	F	AGAGTTTGATCCTGGCTCAG	58	1500
	R	CTACGGCTACCTTGTACGA		
<i>ampC</i>	F	ACTTTCAACAGGTGTGCTGGG	55–57	405
	R	ACCGTACGCATATCGGCTTT		
<i>lysR</i>	F	ATCTGGTCACCGGCATACCT	55–57	303
	R	TCATAGGGTGTGTGGTCACG		
<i>qseB</i>	F	GCAATCCTGACCTCACCT	55–57	407
	R	TCGTCCCAGGTGTAGAGCTT		
<i>qseC</i>	F	TAATGACGGCCAGAACGGAG	55–57	536
	R	GATCCAACGCTTTCTCACGC		

2.7 PCR Amplification

Conventional PCR was performed to detect the presence of target genes (16S rRNA, *ampC*, *lysR*, *qseB*, and *qseC*). Each reaction was carried out in a total volume of 25 µL containing 12.5 µL of 2× PCR Master Mix, 1 µL each of forward and reverse primer, 2 µL of template DNA, and 8.5 µL of nuclease-free water. PCR amplification was performed using a thermal cycler under the following conditions: initial denaturation at 95°C for 5 min, followed by 30–35 cycles of denaturation at 95°C for 30 s, annealing at 55–58°C for 30 s (depending on the primer set), and extension at 72°C for 45–60 s. A final extension step was performed at 72°C for 5 min. Each PCR run included a positive control (a previously confirmed *K. pneumoniae* isolate carrying the respective target gene) and a non-template negative control (sterile nuclease-free water substituted for DNA template) to validate assay specificity and detect potential contamination. Annealing temperatures for each gene-specific primer pair were optimized by gradient PCR before application. Representative amplicons from each target gene were subjected to Sanger sequencing (performed commercially) to confirm PCR product identity; sequence analysis confirmed the expected gene identity for all targets by BLAST alignment against the NCBI nucleotide database.

2.8 Agarose Gel Electrophoresis

PCR products were analyzed by electrophoresis on 1.5% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. A DNA ladder (100–3000 bp) was used as a molecular size marker. Electrophoresis was performed at 80–100 V for 45–60 min. Amplified DNA bands were visualized under UV transillumination and documented using a gel documentation system.

3. Results and Discussion

3.1 Distribution of Bacterial Isolates

A total of 200 clinical samples (sputum and urine) were collected from hospitalized patients. Following culture and incubation, 100 samples showed no bacterial growth, while 100 samples yielded bacterial isolates. Among these, 20 isolates (20%) were identified as *Klebsiella pneumoniae*, whereas the remaining 80 isolates belonged to other bacterial species (Table 2).

Table 2. Distribution of bacterial isolates from clinical samples.

Culture Result	Number of Isolates
No bacterial growth	100
<i>Klebsiella pneumoniae</i>	20
Other bacterial species	80
Total	200

3.2 Distribution of *Klebsiella pneumoniae* According to Sample Source

The distribution of *K. pneumoniae* isolates varied according to specimen source. The majority of isolates were obtained from sputum samples (n = 17), while a smaller number were recovered from urine samples (n = 3) (Table 3).

Table 3. Distribution of *Klebsiella pneumoniae* isolates according to sample source.

Sample Source	Number of Isolates (n = 20)
Sputum	17 (85%)
Urine	3 (15%)
Total	20 (100%)

3.3 Growth Characteristics on MacConkey Agar

Klebsiella pneumoniae isolates showed characteristic growth on MacConkey agar after incubation at 37°C for 24 h. Colonies appeared as large, smooth, pink, and mucoid, confirming lactose fermentation ability (Figure 1). This morphological feature was used as a key preliminary criterion for presumptive identification of *K. pneumoniae*.



Figure 1. Growth of *Klebsiella pneumoniae* on MacConkey agar showing large, smooth, pink mucoid colonies characteristic of lactose fermentation.

3.4 VITEK System Identification

All 20 isolates were identified using the VITEK 2 Compact system (Table 4). Results confirmed all isolates as *Klebsiella pneumoniae* ssp. *pneumoniae* with an identification probability of 99%, indicating high accuracy and reliability of the automated system.

Table 4. Identification of *Klebsiella pneumoniae* isolates using the VITEK 2 Compact system.

Isolate No.	Identified Organism	Probability (%)
1–20	<i>Klebsiella pneumoniae</i> ssp. <i>pneumoniae</i>	99

3.5 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing revealed high levels of resistance among the isolates, particularly to β -lactam antibiotics (Table 5). All isolates demonstrated complete resistance (100%) to cefazolin, cefuroxime, and cefuroxime axetil. High resistance rates were also recorded for ceftazidime and ceftriaxone (90%). Carbapenems showed moderate effectiveness, with susceptibility rates ranging from 55% to 60%. Among non- β -lactam antibiotics, amikacin exhibited the highest activity (85% susceptibility), whereas high resistance rates were observed for nitrofurantoin (85%) and trimethoprim/sulfamethoxazole (80%).

Table 5. Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolates (n = 20).

Antibiotic	S (%)	I (%)	R (%)
Amoxicillin/Clavulanic acid	25	10	65
Piperacillin/Tazobactam	55	0	45
Cefazolin	0	0	100
Cefuroxime	0	0	100
Cefuroxime axetil	0	0	100
Ceftazidime	10	0	90
Ceftriaxone	10	0	90
Cefepime	20	15	65
Imipenem	55	20	25
Meropenem	60	0	40
Ertapenem	60	10	30
Amikacin	85	15	0
Gentamicin	55	10	35
Ciprofloxacin	20	15	65
Fosfomycin	50	20	30
Nitrofurantoin	5	10	85
Trimethoprim/Sulfamethoxazole	20	0	80

S, susceptible; I, intermediate; R, resistant.

3.6 Molecular Identification

3.6.1 Detection of 16S rRNA Gene

The 16S rRNA gene was amplified in all 20 *K. pneumoniae* isolates using conventional PCR. A clear amplification product was observed at approximately 1500 bp in all lanes (1–20), consistent with the expected product size (Figure 2). The 100% detection rate confirms the accuracy of bacterial identification before further molecular analysis.

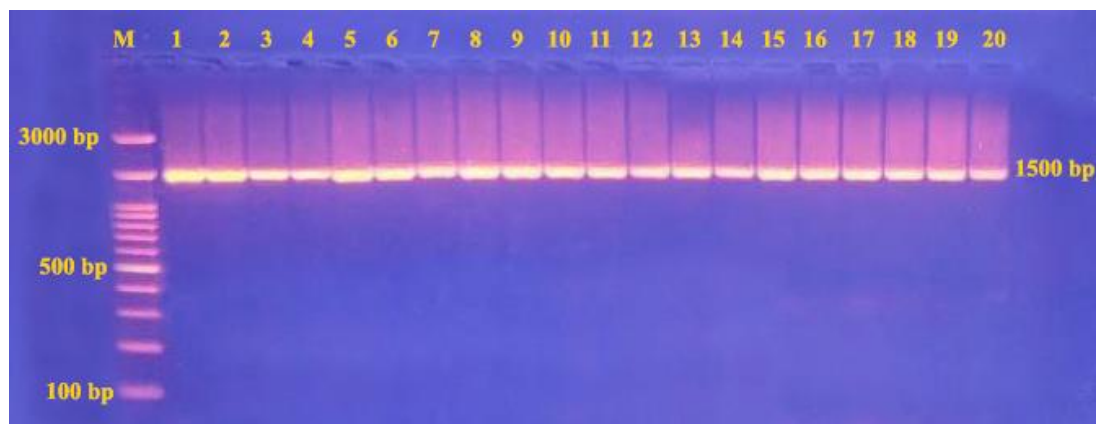


Figure 2. Agarose gel electrophoresis of PCR amplification of the 16S rRNA gene in *Klebsiella pneumoniae* isolates (n = 20). Lane M: DNA ladder (100–3000 bp). A band at ~1500 bp was observed in all lanes (1–20), indicating 100% detection rate.

3.6.2 Detection of the *AmpC* Gene

PCR amplification of the *ampC* gene produced a product at approximately 405 bp, consistent with the expected size. Positive amplification was observed in isolates 3, 6, 10, 11, 12, 16, 18, and 19, indicating that 8 of 20 isolates (40%) carried the *ampC* gene (Figure 3). The remaining 12 isolates (60%) were negative, suggesting that resistance in these isolates may be due to mechanisms not tested.

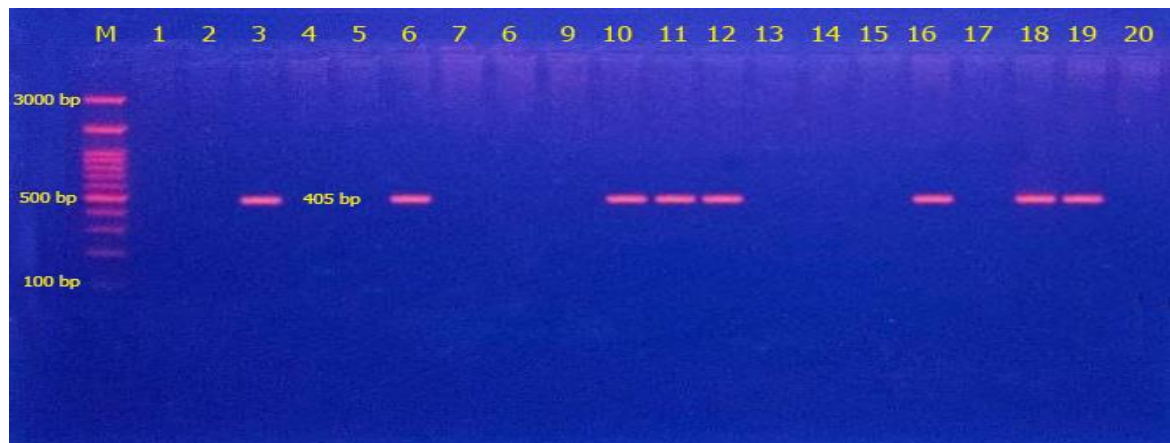


Figure 3. Agarose gel electrophoresis of PCR amplification of the *ampC* gene. Lane M: DNA ladder (100–3000 bp). Positive bands (~405 bp) were observed in lanes 3, 6, 10, 11, 12, 16, 18, and 19, representing 40% of the isolates.

3.6.3 Detection of the *lysR* Gene

The *lysR* gene was detected in all 20 isolates (100%), producing a consistent band at approximately 303 bp (Figure 4). The universal prevalence of this gene across all clinical isolates indicates its likely conserved and essential regulatory function in *K. pneumoniae*.

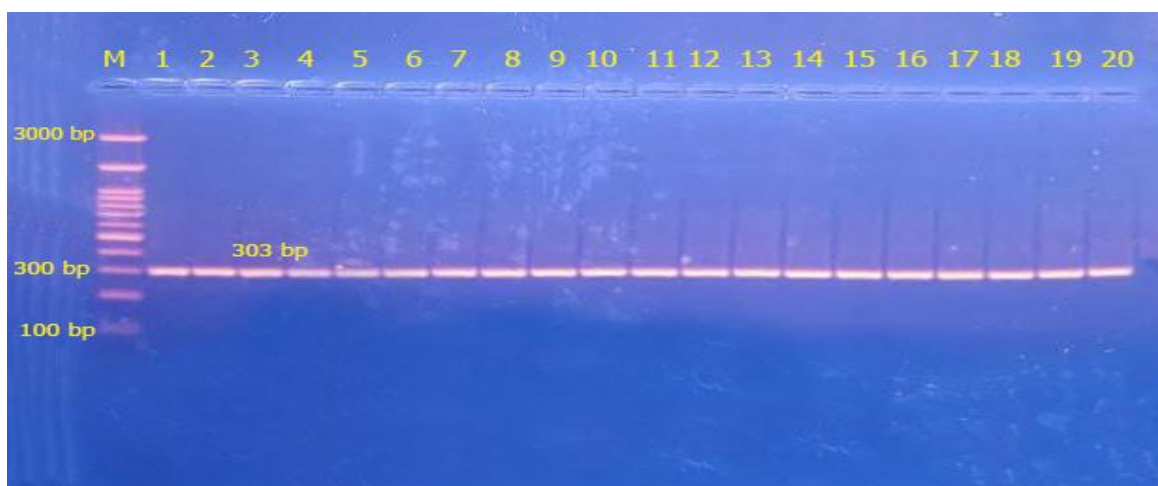


Figure 4. Agarose gel electrophoresis of PCR amplification of the *lysR* gene. Lane M: DNA ladder (100–3000 bp). A band at ~303 bp was observed in all lanes (1–20), indicating 100% prevalence.

3.6.4 Detection of *qseB* Gene

The *qseB* gene was detected in all 20 isolates (100%), producing a consistent amplification band at approximately 407 bp (Figure 5). This complete prevalence suggests that the QseB/QseC two-component signaling system is a constitutive feature of *K. pneumoniae* across clinical sources.

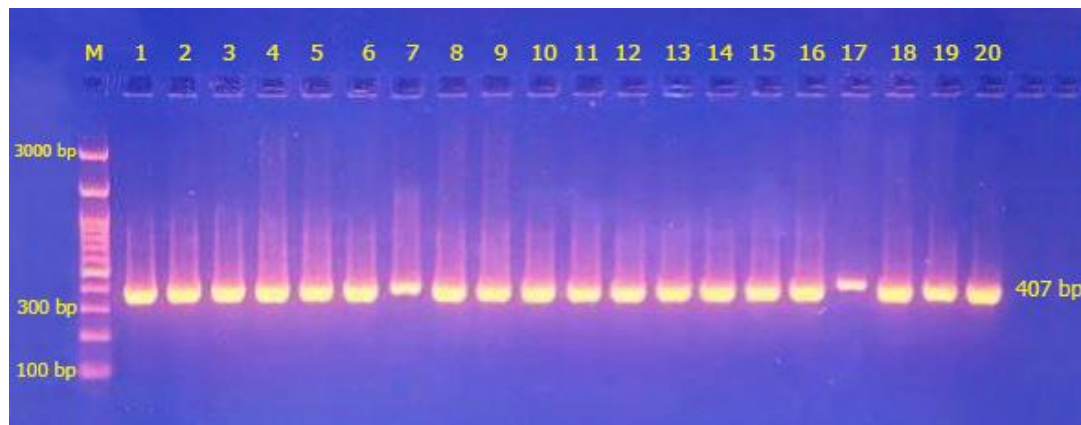


Figure 5. Agarose gel electrophoresis of PCR amplification of the *qseB* gene. Lane M: DNA ladder (100–3000 bp). A band at ~407 bp was observed in all lanes (1–20), confirming 100% prevalence.

3.6.5 Detection of *qseC* Gene

PCR amplification of the *qseC* gene produced a band at approximately 536 bp. Positive amplification was observed in 17 of 20 isolates (85%), specifically in lanes 1–6, 8–16, 18, and 20, while isolates 7, 17, and 19 showed no amplification (Figure 6). This partial absence may reflect allelic diversity or gene deletion in a subset of the clinical isolates.

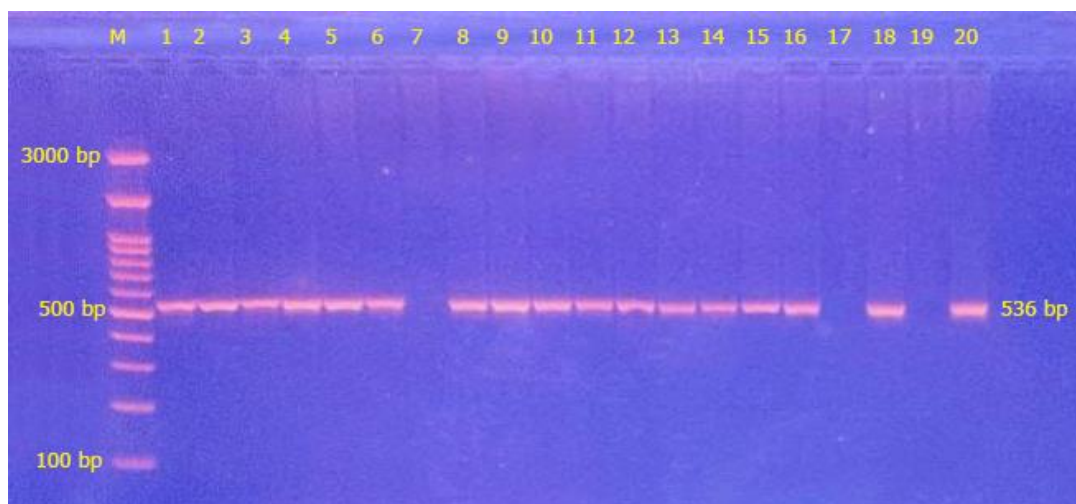


Figure 6. Agarose gel electrophoresis of PCR amplification of the *qseC* gene. Lane M: DNA ladder (100–3000 bp). A band at ~536 bp was observed in lanes 1–6, 8–16, 18, and 20. Lanes 7, 17, and 19 showed no amplification, yielding an 85% detection rate.

3.7 Discussion

The results of the present study indicate that *Klebsiella pneumoniae* isolates were more frequently obtained from sputum samples (85%) than from urine samples (15%), reflecting the significant role of this bacterium in hospital-acquired respiratory tract infections. This distribution pattern is consistent with the nature of this organism as a major opportunistic pathogen among hospitalized patients, particularly those who are immunocompromised or receiving mechanical ventilation. Mohamed and Al-Taai [16] reported similarly high isolation rates of this bacterium from sputum samples in nosocomial infections, supporting the current findings and highlighting its strong capacity for respiratory colonization. Furthermore, the ability of *K. pneumoniae* to form biofilms significantly contributes to its persistence within the respiratory tract and on medical surfaces, as demonstrated by Hussein et al. [17], who linked biofilm formation with increased colonization and antibiotic resistance. Natural plant extracts, including those from *Piper* species, have demonstrated bactericidal properties and significant biofilm-inhibitory activity against pathogenic bacteria [18]. Nanomaterial-based approaches, such as green-synthesized ZnO-TiO₂ nanoparticles, have similarly

exhibited potent antibacterial effects [19]. Host nutritional and immune status also modulate susceptibility to bacterial infections in diverse biological systems [20]. GC-MS profiling of plant-derived bioactive compounds has revealed constituents with relevant biological activities [21], while insect-derived antimicrobial peptides represent a promising class of novel antimicrobial agents [22]. Natural bioactive compounds such as berberine have demonstrated significant biomedical effects in experimental models [23], and the intersection of antimicrobial resistance with other disease contexts underscores the breadth of drug-resistance challenges in modern healthcare [24]. The antimicrobial susceptibility results revealed a high level of resistance, particularly against β -lactam antibiotics, with complete resistance to cephalosporins and high resistance to ceftriaxone and ceftazidime. This finding reflects strong selective pressure due to the widespread use of these antibiotics in clinical settings. These results are consistent with those reported by Abdulhasan et al. [25], who demonstrated a high prevalence of ESBL genes such as *blaSHV* and *blaCTX-M* in *K. pneumoniae* isolates, which play a key role in cephalosporin inactivation. Similarly, Albassam [26] reported high resistance rates to β -lactam antibiotics, indicating that this phenomenon is not localized but part of a global trend. Moreover, Philippon et al. [27] highlighted that AmpC enzymes can become a major source of resistance when overexpressed, thereby enhancing their ability to hydrolyze a broad spectrum of β -lactam antibiotics.

Regarding other antibiotic classes, carbapenems showed only moderate effectiveness, a concerning sign of emerging resistance to this critical class of last-resort antibiotics. This observation is consistent with that of Badamchi et al. [28], who emphasized that the inappropriate use of carbapenems accelerates the emergence of resistant strains, posing a serious public health threat. In addition, Realegeno et al. [29] demonstrated that the combination of AmpC production and porin loss can confer carbapenem resistance even in the absence of carbapenemases, which may explain the reduced susceptibility observed in this study. In contrast, amikacin exhibited the highest susceptibility rate, suggesting that it remains relatively effective against these isolates, possibly attributed to its lower susceptibility to enzymatic degradation and its relatively limited clinical use. At the molecular level, all isolates were positive for the 16S rRNA gene, confirming the accuracy of molecular identification. Monawer [30] confirmed that 16S rRNA serves as a gold standard for accurate identification of *K. pneumoniae*, while Kumar et al. [31] emphasized its importance in molecular epidemiology and bacterial classification.

The detection of the *ampC* gene in 40% of the isolates falls within the range reported in several regional studies, although there are clear geographical variations. Abdulhasan et al. [25] reported a lower prevalence of 16.7%, whereas Jomehzadeh et al. [32] reported a prevalence of 36.3%, indicating variability in gene distribution across different populations. Furthermore, Etemadi et al. [33] noted that AmpC detection can be challenging due to its inducible nature, which may result in underexpression under certain conditions and consequently hinder its detection by conventional PCR. The study also demonstrated the universal presence of the *lysR* gene in all isolates, indicating its essential role in regulating various bacterial processes, including virulence and environmental stress response. Srinivasan et al. [34] showed that LysR plays a key role in antimicrobial resistance regulation, while Yang et al. [35] reported that deletion of a LysR family gene led to a significant reduction in virulence and biofilm formation, highlighting its importance in bacterial survival within the host. Additionally, Baugh et al. [36] described the LysR family as one of the largest regulatory families in bacteria, controlling multiple cellular functions.

Regarding the QseB/QseC system, the high prevalence of these genes suggests their important role in regulating bacterial behavior. This two-component system responds to environmental cues and regulates gene expression related to virulence, motility, and biofilm formation. Zhu et al. [37] described the QseBC system as a global regulator of bacterial behavior controlling multiple pathways associated with infection. Similarly, Lv et al. [38] demonstrated that disruption of QseC leads to increased biofilm formation and enhanced virulence in hypervirulent *K. pneumoniae*, reflecting the complex regulatory role of this system. By integrating antimicrobial resistance results with molecular findings, it becomes evident that *K. pneumoniae* isolates in this study possess a combination of resistance mechanisms and regulatory factors that enhance their ability to adapt and survive within the host. The coexistence of resistance genes, such as *ampC*, with regulatory genes, such as *lysR* and *qseBC*, may lead to increased expression of virulence factors and enhanced biofilm formation, thereby increasing bacterial tolerance to antibiotics. This has been supported by recent studies that have

highlighted the link between quorum-sensing systems and antimicrobial resistance [39,40]. A clear discrepancy was observed between the phenotypic resistance pattern and the molecular detection of the *ampC* gene: all isolates exhibited high β -lactam resistance despite *ampC* being detected in only 40% of them. This phenomenon reflects the complex, multifactorial nature of the resistance mechanisms in this bacterium. It can be explained by the presence of other β -lactamase genes—such as ESBLs, widely distributed as reported by Abdulhasan et al. [25]—capable of conferring resistance even in the absence of *AmpC*, and by the co-occurrence of these genes on transferable plasmids that facilitate rapid dissemination [41]. This discrepancy may also be attributed to the presence of different *AmpC* gene types not investigated in this study (e.g., CIT, DHA, CMY), which show significant diversity across clinical isolates [32,42].

Moreover, regulatory mutations play a critical role in enhancing *AmpC* expression. Alterations in regulatory genes such as *ampR*, *ampD*, and *ampG* may lead to hyperproduction of this enzyme. Barceló et al. [43] demonstrated that disruption of the peptidoglycan recycling pathway results in *AmpC* overproduction and increased resistance, while Li et al. [44] confirmed the essential role of *ampG* in regulating inducible *AmpC* expression. Non-enzymatic mechanisms such as porin loss and increased efflux pump activity reduce intracellular antibiotic concentrations. Biofilm formation also acts as a protective barrier that limits antibiotic penetration [39], and quorum-sensing systems such as QseBC contribute to the regulation of these processes and to the enhancement of virulence, thereby increasing the overall complexity of resistance [40]. Therefore, the observed discrepancy between phenotypic and molecular findings in this study should not be considered contradictory but rather a reflection of the complex and multifactorial nature of antimicrobial resistance in *K. pneumoniae*, making this bacterium one of the most challenging pathogens in modern clinical environments.

4. Conclusions

The results of this study demonstrated a high prevalence of antibiotic resistance among *Klebsiella pneumoniae* clinical isolates from Al-Hillah, Iraq, particularly against β -lactam antibiotics. Although the *ampC* gene was detected in only 40% of isolates, the widespread phenotypic resistance observed suggests the involvement of additional mechanisms such as ESBLs and other resistance determinants. The universal detection of the 16S rRNA gene confirmed accurate molecular identification, while the 100% prevalence of *lysR* and *qseB* genes, along with the 85% prevalence of *qseC*, suggests a potential role for regulatory systems in bacterial adaptation and virulence. Overall, this study highlights the complexity of antimicrobial resistance in *K. pneumoniae* and underscores the need for combined molecular and phenotypic approaches for accurate detection and effective infection control.

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