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Original Research

Detection of Some β-Lactamase and Aminoglycoside Resistance Genes Among Local Multiple Antibiotic Resistance *Klebsiella pneumoniae* Isolates in Najaf, Iraq

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Abstract

Klebsiella pneumoniae is an opportunistic pathogen responsible for a wide range of healthcare-associated infections. It is frequently linked to resistance to multiple antibiotic classes and can persist in hospital environments despite infection control measures. This study aimed to evaluate the distribution of antibiotic resistance genes among *K. pneumoniae*. A total of 38 isolates were obtained from different clinical specimens and identified using the VITEK 2 Compact (GN-ID Card) and PCR targeting 16S rRNA. Multiple antibiotic-resistant isolates were characterized using the disc diffusion method. Resistance genes were detected by PCR, including *blaTEM*, *blaSHV*, *blaPER*, *ctx-M1, ctx-M9, aph(3')-Ia*, *aac(3')-Ia*, *aac(3')-IIa*, *aac(3')-Iva*, *aac(6')-Ib*, *ant(2'')-Ia*, *ant(4')-IIa*. Results revealed a wide distribution of resistance genes. Among β-lactamase genes, *blaTEM* was predominant, detected in 32 isolates (84.2%), compared with *blaSHV* in 21 isolates (55.3%), while *blaPER* was absent. Cephalosporin resistance genes included *ctx-M1*, *ctx-M9* and *aph(3')-Ia, which* were detected in 15 (39.5%), 8 (21.1%), and 10 (26.3%) isolates, respectively. Aminoglycoside resistance genes were also prevalent: *aac(3')-Ia* (76.3%), *aac(3')-IIa* (44.7%), and *aac(3')-Iva* (36.8%). Other genes included *ant(2'')-Ia* (42.1%), *ant(4')-IIa* (15.8%), and *aac(6')-Ib* (10.5%) were detected. The high prevalence of multidrug resistance highlights the correlation

between phenotypic and genotypic traits. Resistance may also be associated with phenotypes such as capsule production or biofilm formation, rather than solely with resistance genes.

Keywords
Klebsiella pneumoniae; antibiotic resistance; β-lactamase; aminglycoside resistance; polymerase chain reaction

1. Introduction

Klebsiella pneumoniae, a clinically important member of the Enterobacteriaceae, is one of the leading causes of healthcare-associated infections, particularly in intensive care units. It contributes to the spread of virulence factors and antimicrobial resistance (AMR), with more than 100 distinct acquired resistance genes reported [1, 2]. In recent years, antibiotic resistance among Gram-negative and Gram-positive bacteria, including *K. pneumoniae*, has become a major challenge for healthcare systems [3-6]. Two major types of resistance are commonly observed: (i) Extended-spectrum β-lactamase (ESBL) production, conferring resistance to cephalosporins and monobactams. (ii) Carbapenemase production, conferring resistance to carbapenems and most other β-lactams [3].

K. pneumoniae has two primary types of antibiotic resistance: (i) Bacteria that express extended-spectrum β-lactamase (ESBL) develop resistance to cephalosporins and monobactams, (ii) Bacteria resistant to carbapenems and most other β-lactams develop carbapenemases [7]. β-lactamases, which belong to the CTX-M, TEM, SHV, and OXA families, have distinct hydrolytic characteristics and are often encoded by the *bla* genes, such as *blaSHV-1,* which encodes a penicillinase that imparts innate resistance to ticarcillin, ampicillin, and amoxicillin. Also, *K. pneumoniae* has been a major species that can acquire plasmids coding for class C Ambler β-lactamase, or AmpC-type cephalosporinase, which hydrolyzes most β-Lactam antibiotic such as penicillins, third-generation cephalosporins, and monobactams; such enzymes are not efficiently inhibited by the traditional ESBL inhibitors [8, 9]. The CTX-M family exhibits more activity against cefotaxime than ceftazidime (third-generation cephalosporins) due to the antibiotic binding site's shape, which enables effective recognition of cefotaxime but not ceftazidime (a bigger molecule) [10]. The high frequency of *blaCTX-M* among isolates may reflect selective pressure the wide spread prescription of third-generation cephalosporins, mainly cefotaxime and ceftriaxone, in various geographical regions, including in Iraq [11-13]. In Iraq, Several reports showed that *blaCTX-M* was the most prevalent gene among ESBL-producing *K. pneumoniae* [14-16]. In Najaf, Iraq, most carbapenem-resistant *K. pneumonia* (CRKP) local isolates showed high rates of multidrug resistance (MDR), with high prevalence of carbapenemase genes among isolates, where *blaOXA-51*, and *blaNDM* are the most common genes [16]. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [17].

Aminoglycosides, once considered a cornerstone therapy for multidrug-resistant pathogens, have lost efficacy due to many resistance mechanisms, such as aminoglycoside nucleotide-transferase (ANT), aminoglycoside phosphor-transferase (APH), and aminoglycoside acetyl-

transferase (AAC), as well as target site mutations and overexpression of efflux pumps [18, 19]. Genes encoding aminoglycoside acetyltransferases, such as *aac(3')-II and aac(6')-Ib*, are among the most prevalent in *K. pneumoniae*. Additionally, 16S rRNA methyltransferases (ArmA, RmtB, RmtC), often carried on mobile genetic elements, confer high-level resistance and are frequently co-transmitted with ESBLs and carbapenemases [20]. Such genes encoding AAC and 16S rRNA methylases (ArmA, RmtB, and RmtC) are transported to other organisms, resulting in the establishment of multidrug-resistant [21]. Given the widespread occurrence of multidrug-resistant *K. pneumoniae*, this study aimed to investigate the prevalence of antibiotic resistance genes among clinical isolates in Najaf, Iraq.

2. Materials and Methods

2.1 Study Design and Bacterial Isolates

Thirty-eight *K. pneumoniae* isolates were collected from different clinical sources, including urine, burn wounds, nasopharyngeal swabs, vaginal swabs, and fluid extraction devices of patients who were admitted to a private laboratory in Najaf City/Iraq. Identification was performed using biochemical tests and the VITEK 2 Compact system (bioMérieux, France) using the GN-ID Card. All steps of the research are summarized in Figure 1.

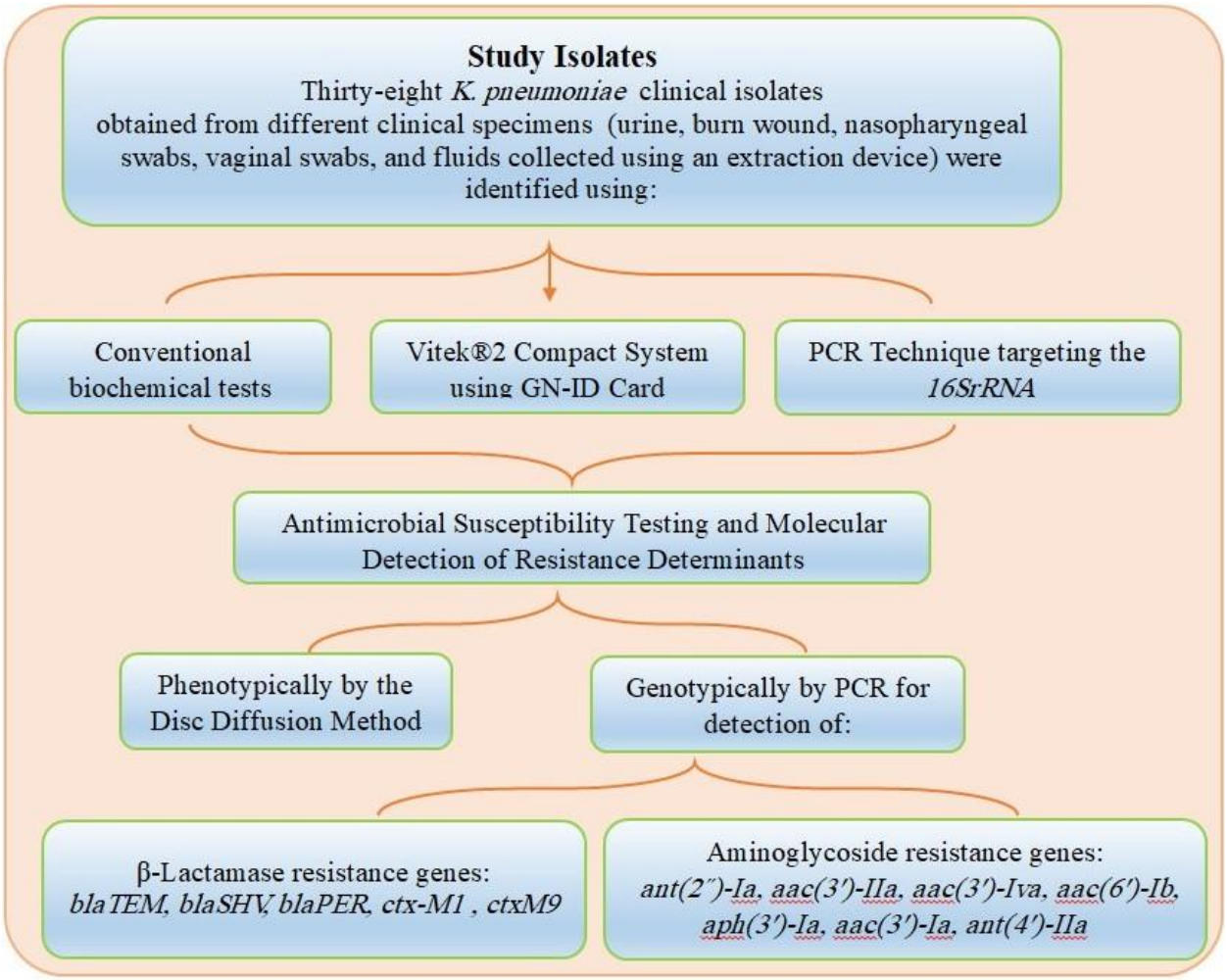

Figure 1 Summerization of research steps.

2.2 Phenotypic Detection of Antibiotic Resistance

Antibiotic resistance was assessed using the Kirby-Bauer disc diffusion method [22]. Nine antibiotics were tested: Amoxilav (AMC, 30 µg/discs), Meropenem (MEM, 10 µg/discs), Tobramycin (TOB, 10 µg/discs), Aztreonam (ATM, 30 µg/discs), Azithromycin (AZM, 15 µg/discs), Doxycycline (DO, 30 µg/discs), Tetracycline (TE, 30 µg/discs), Nalidixic acid (NA, 30 µg/discs), and Ofloxacin (OFX, 5 µg/discs). The antibiotic resistance patterns were determined according to the Clinical Laboratory Institution Standard, 2025 [23].

2.3 Genotypic Detection of Antibiotic Resistance

2.3.1 DNA Extraction and Isolation

DNA was extracted using the alkaline lysis method [24]. Briefly, Cells were resuspended in glucose buffer (25 mM Tris base, 10 mM EDTA, and 50 mM Glucose), lysed with NaOH/SDS (0.2 M NaOH in 1% of Sodium dodecyl sulfate), neutralized with sodium acetate buffer (60 mL of 5 mM of sodium acetate, 11.5 ml of glacial acetic acid and 28.5 ml of distilled water, pH 5.8), and precipitated with isopropanol (0.6 ml/1 ml v/v). DNA was washed with 70% ethanol, re-suspended in TE buffer (Magen/China), and stored at -20°C.

2.3.2 PCR Technique

Monoplex and multiplex PCR were performed using primers listed in Table 1. PCR mixture of monoplex consist of 4 µl of Master mix (Solis Biodyne/Estonia), 0.4 µl of each forward and reverse, 14.4 µl of nuclease free water, and 0.8 µl DNA template, while the PCR mixture of multiplex consist of 4 µl of Master mix (Solis Biodyne/Estonia), 0.4 µl of each forward and reverse for each genes, 12.8 µl of nuclease free water, and 0.8 µl DNA template. PCR conditions included initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C, 30 s), annealing (Table 1), and elongation (72°C, 2 min), with a final extension at 72°C for 10 min. Amplification takes place in thermocycler (Biometra, U.S.A).

Table 1 The sequences of oligo-synthesize primers (Macrogen, Korea).

Genes Target	Sequences (5′-3′)	Size (bp)	Annealing (°C/sec)	Reference
16S rRNA	F: ATTTGAAGAGGTTGCAAACAT R: TTCTGAAGTTTTCTTGTGTTC	130	50/60	[25]
blaTEM	F: TGCGGTATTATCCCGTGTTG R: TCGTCGTTTGGTATGGCTTC	296	54/30	[26]
blaSHV	F: AGCCGCTTGAGCAAATTAAAC R: ATCCCGCAGATAAATCACCAC	713		
blaPER	F: TGGGCTTAGGGCAGAAAG R: GAATACCTGGGCTCCGATAA	607		[27]
ctx-M1	F: CTCACGCTGTTGTTAGGAA R: ACGGCTTTCTGCCTTAGGTT	780	56/30	[28]
ctx-M9	F: ATGGTGACAAAGAGAGTGCA R: CCCTTCGGCGATGATTCTC	863		[29]

ant(2'')-Ia	F: ATCTGCCGCTCTGGAT R: CGAGCCTGTAGGACT	404	58/30	[30]
aac(3')-IIa	F: ATGCATACGCGGAAGGC R: TGCTGGCACGATCGGAG	822	53/30	[30]
aac(3')-Iva	F: GTGTGCTGCTGGTCCACAGC R: AGTTGACCCAGGGCTGTCGC	627		[31]
aac(6') -Ib	F: ATGACTGAGCATGACCTTG R: AAGGGTTAGGCAACACTG	524		[30]
aph(3')-Ia	F: CGAGCATCAAATGAAACTGC R: GCGTTGCCAATGATGTTACAG	623	56/30	[32]
aac(3')-Ia	F: GACATAAGCCTGTTCGGTT R: CTCCGAACTCACGACCGA	372	58/30	[32]
ant(4')-IIa	F: ATCGTCTGCGAGAAGCGTAT R: TAAAACGCCTATCCGTCACC	839		[33]

2.3.3 Electrophoresis Technique

PCR products were analyzed by agarose gel electrophoresis (1% agarose in TBE buffer (1×), stained with ethidium bromide (0.05 µg/ml; Himedia/India)). Gels were run in an electrophoresis unit (Biometra/U.S.A) at 80 V for 1 hr. and visualized using a gel documentation system (Biometra, U.S.A).

3. Results

3.1 Identification of Klebsiella pneumoniae

PCR amplification of 16S rRNA confirmed that all 38 isolates belonged to *K. pneumoniae* by the appearance of an amplicon of 130 bp (Figure 2).

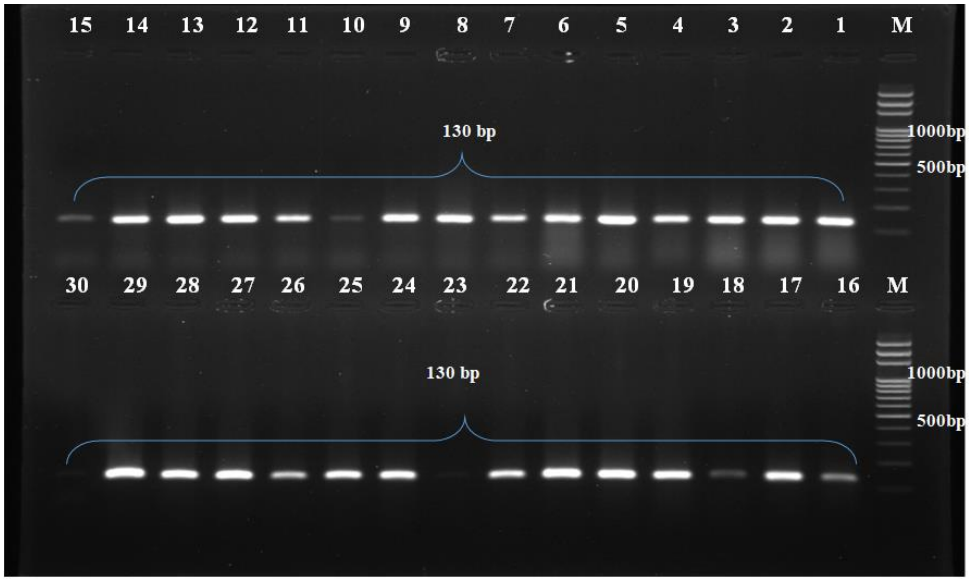

Figure 2 Agarose gel electrophoresis (1% Agarose at 80 Volt for 1 hr.) of *16Sr RNA* amplicon (130 bp) of *K. pneumoniae.* Line M: DNA Ladder 100 bp (Solis Biodyne/Estonia), Line 1-30: positive results for amplification.

3.2 Phenotypic Detection of Antibiotic Resistance Pattern

Antibiotic susceptibility testing revealed high ates of resistance. All isolates (100%) were resistant to amoxicillin/clavulanate. Moderate resistance was observed to aztreonam and nalidixic acid (60.5% each). Resistance to tetracycline (47.4%) and ofloxacin (44.7%) was also notable. Lower resistance rates were recorded for doxycycline (39.5%), tobramycin (28.9%), meropenem (21%), and azithromycin (15.8%) (Figure 3). All isolates were classified as MDR due to resistance to one antibiotic in more than three categories.

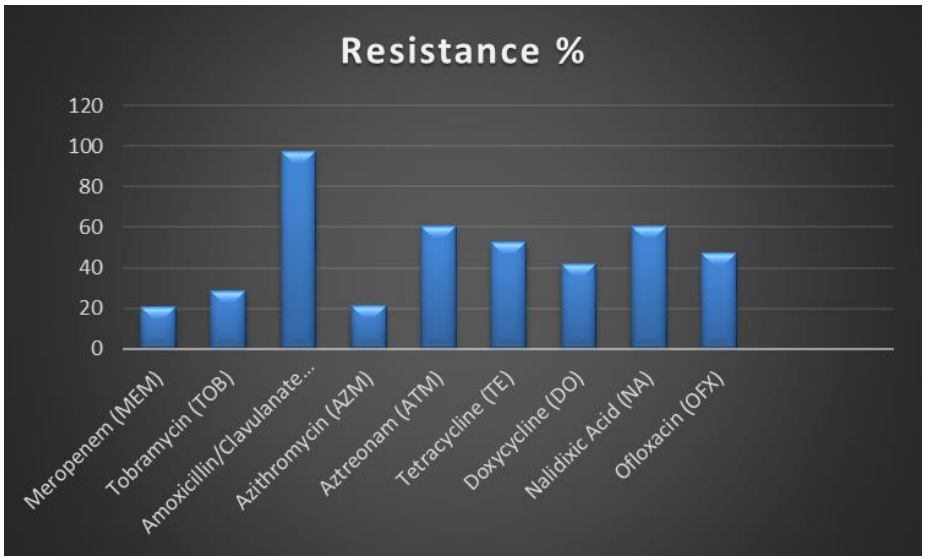

Figure 3 The percentage of antibiotic resistance of *K. pneumoniae*.

3.3 Genotypic Detection of β-Lactam Coding Genes

PCR amplification revealed that *blaTEM* (296 bp) was the most prevalent gene, detected in 32 isolates (84.2%). *blaSHV* (713 bp) was present in 21 isolates (55.3%), while *blaPER* (607 bp) was absent in all isolates (Figure 4).

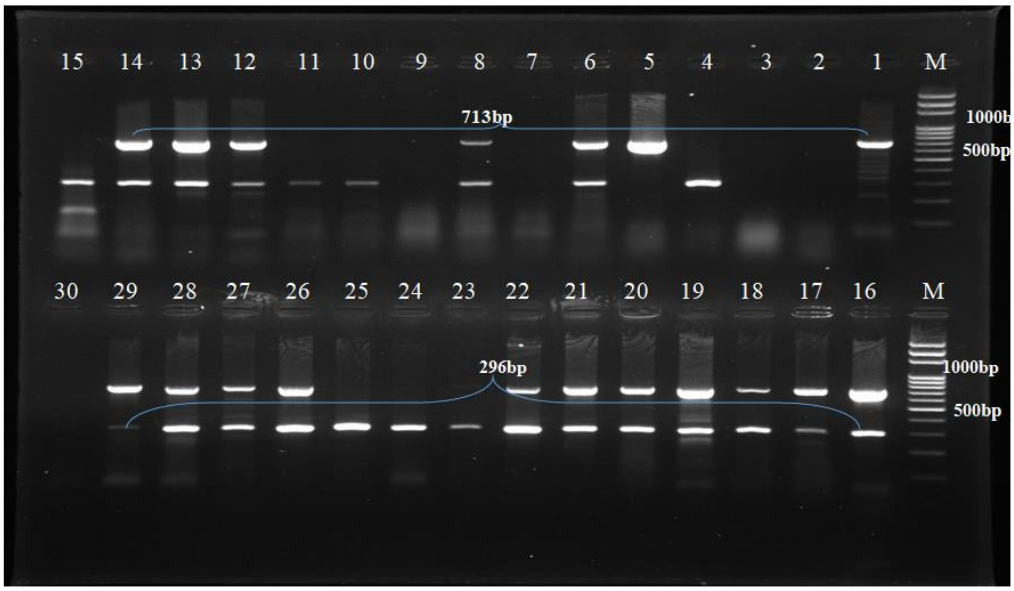

Figure 4 Ethidium bromide stain agarose gel electrophoresis (1% Agarose at 80 Volt for 1 hr.) of *blaTEM* (296 bp) and *blaSHV* (713 bp), and *blaPER* (607 bp) amplicons in *K. pneumoniae*. Line M: DNA Ladder 100 bp (Solis Biodyne/Estonia), Line 1-30: positive and negative results for amplification.

3.4 Genotypic Detection of Cephalosporin Coding Genes

Amplification of cephalosporin resistance genes showed that 15 isolates (39.5%) carried *ctx-M1* (780 bp), 8 isolates (21.1%) carried *ctx-M9* (863 bp), and 10 isolates (26.3%) carried *aph(3')-Ia* (623 bp) (Figure 5).

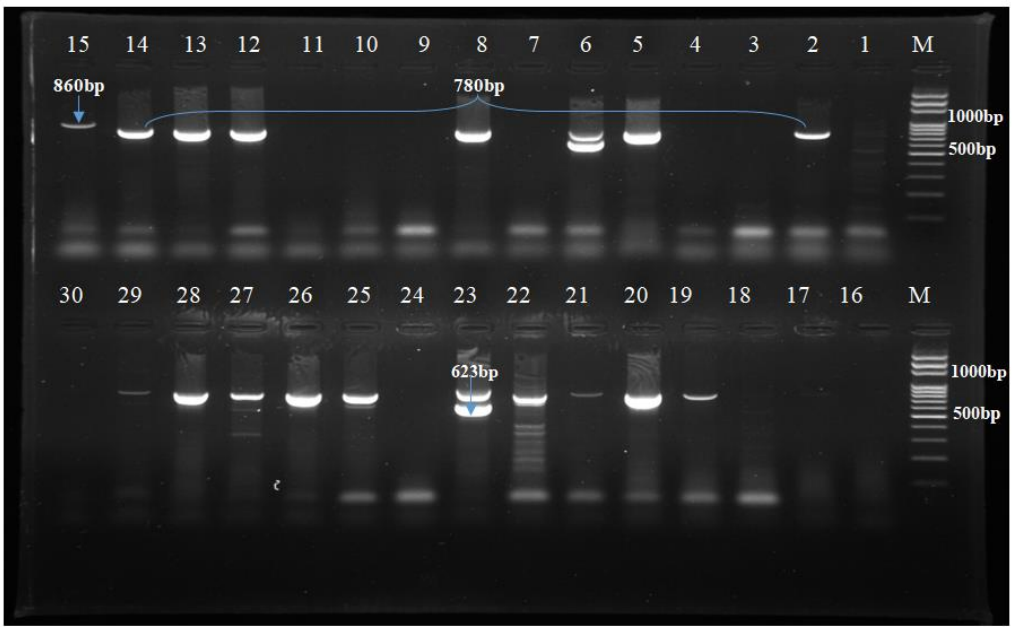

Figure 5 Ethidium bromide stain agarose gel electrophoresis (1% Agarose at 80 Volt for 1 hr.) of *ctx-M1* (780 bp) and *ctx-M9* (863 bp), and *aph(3')-Ia* (623 bp) amplicons in *K. pneumoniae.* Line M: DNA Ladder 100 bp (Solis Biodyne/Estonia), Line 1-30: positive and negative results for amplification.

3.5 Detection of Aminoglycoside Coding Gene

Among aminoglycoside resistance genes, *aac(3')-Ia* (372 bp) was detected in 29 isolates (76.3%), *aac(3')-IIa* (822 bp), in 17 isolates (44.7%), and *aac(3')-Iva* (627 bp) in 14 isolates (36.8%). Other genes included *ant(2'')-Ia* (16 isolates, 42.1%), *ant(4')-IIa* (6 isolates, 15.8%), and *aac(6')-Ib* (4 isolates, 10.5%) were also observed (Figure 6 and Figure 7).

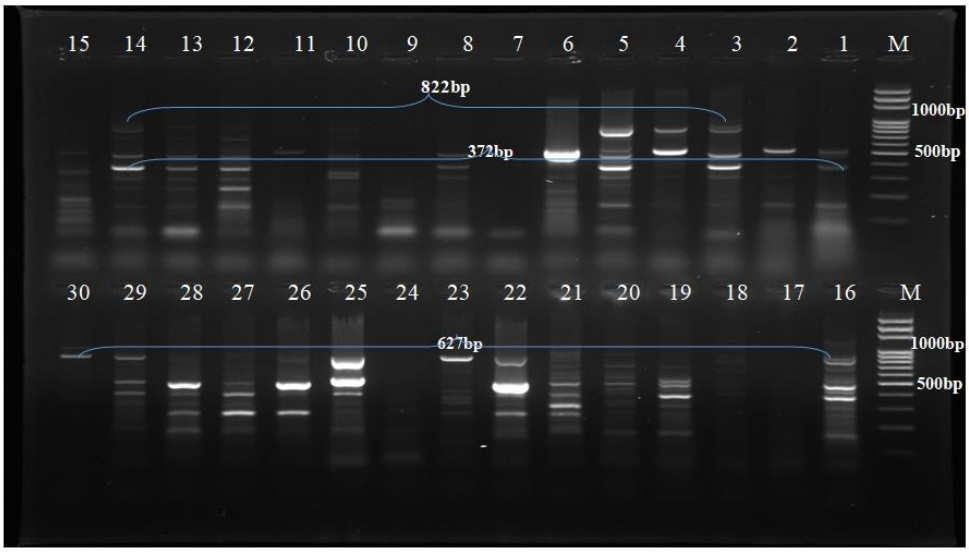

Figure 6 Ethidium bromide stain garose gel electrophoresis (1% Agarose at 80 Volt for 1 hr.) of *aac(3')-Ia* (372 bp) and *aac(3')-IIa* (822 bp), and *aac(3')-Iva* (627 bp) amplicons in *K. pneumoniae.* Line M: DNA Ladder 100 bp (Solis Biodyne/Estonia), Line 1-30: positive and negative results for amplification.

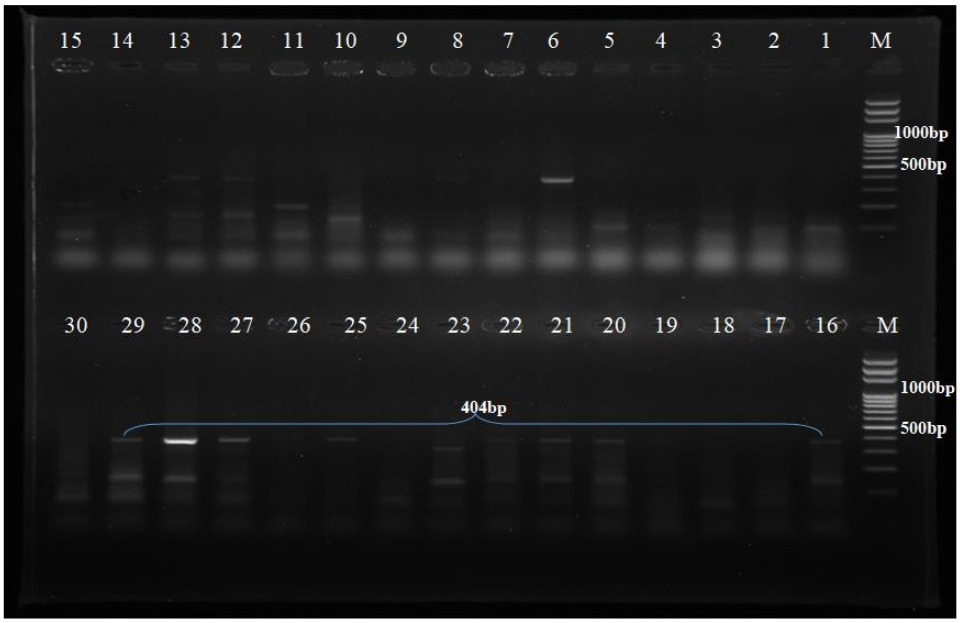

Figure 7 Ethidium bromide stain agarose gel electrophoresis (1% Agarose at 80 Volt for 1 hr.) of *ant(2'')-Ia* (404 bp), *ant(4')-IIa* (839 bp), and *aac(6')-Ib* (524 bp) amplicons in *K. pneumoniae.* Line M: DNA Ladder 100 bp (Solis Biodyne/Estonia), Line 1-30: positive and negative results for amplification.

3.6 The Genetic Contents of Antibiotic Resistance Genes

Analysis of genetic profiles revealed diverse combinations of resistance determinants. For example, 4 isolates carried the pattern *blaTEM, blaSHV, ctx-M1, aac(3')-Ia,* and *ant(2'')-Ia,* while others exhibited variations including *aac(3')-IIa* or *aac(3')-Iva*. Correlation between phenotypic and genotypic resistance was observed. However, some isolates resistant to β-lactams lacked detectable β-lactamase genes, suggesting alternative mechanisms such as capsule formation, biofilm production, porin modification, or altered penicillin-binding proteins (Table 2).

Table 2 Distribution of antibiotic resistance determinant genes among *K. pneumoniae*.

Group	Antibiotic Resistance Gene Pattern	NO. (%) of isolates
I	*blaTEM, blaSHV, blaPER, ctx-M1, ctx-M9, ant(2'')-Ia, aac(3')-IIa, aac(3')-Iva, aac(6')-Ib, aph(3')-Ia, aac(3')-Ia, ant(4')-IIa*	-
II	*blaTEM, blaSHV, ctx-M1, ctx-M9, aac(3')-Ia, aac(3')-Iva, ant(2'')-Ia*	1 (2.6%)
III	*blaTEM, ctx-M1, ctx-M9, aph(3')-Ia, aac(3')-Ia, aac(3')-Iva, ant(2'')-Ia*	1 (2.6%)
IV	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, aac(3')-Iva, ant(2'')-Ia*	2 (5.3%)
V	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, aac(3')-IIa, ant(2'')-Ia*	2 (5.3%)
VI	*blaTEM, ctx-M1, aph(3')-Ia, aac(3')-Ia, aac(3')-Iva, ant(2'')-Ia*	1 (2.6%)
VII	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, ant(2'')-Ia*	4 (10.5%)
VIII	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, aac(3')-IIa*	1 (2.6%)
IX	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, aac(3')-IIa*	1 (2.6%)
X	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia, aac(3')-Iva*	1 (2.6%)
XI	*blaTEM, blaPER, blaSHV, CTX-M1, ant(2'')-Ia*	1 (2.6%)
XII	*blaTEM, blaSHV, aac(3')-Ia, ant(4')-IIa, aac(6')-Ib*	1 (2.6%)
XIII	*blaTEM, blaSHV, aac(3')-Ia, ant(4')-IIa, ant(2'')-Ia*	1 (2.6%)
XIV	*blaTEM, blaSHV, ctx-M1, aac(3')-Ia*	1 (2.6%)
XV	*blaTEM, blaSHV, ctx-M1, aac(3')-IIa*	1 (2.6%)
XVI	*blaTEM, CTX-M9, aph(3')-Ia, aac(3')-Ia*	1 (2.6%)
XVII	*blaTEM, blaSHV, aac(3')-Ia, ant(2'')-Ia*	1 (2.6%)
XVIII	*blaTEM, aac(3')-Ia, aac(3')-IIa*	1 (2.6%)
XIX	*blaTEM, aac(3')-Ia, ant(2'')-Ia*	1 (2.6%)
XX	*blaTEM, blaSHV, ant(4')-IIa*	1 (2.6%)
XXI	*blaTEM, ant(4')-IIa, aac(6')-Ib*	1 (2.6%)

4. Discussion

Klebsiella pneumoniae, a member of the Enterobacteriaceae, is part of the normal gastrointestinal microbiota of healthy humans and animals and can also be found in the skin, nose, throat, and intestinal tract [2]. The 16S rRNA sequences, widely used for the identification of *K. pneumoniae*, possess specific characteristics that make them suitable as global indicators of evolutionary relationships [34, 35].

Antibiotic resistance in *K. pneumoniae* poses a significant global public health challenge. The dominant mechanism of resistance involves the production of β-lactamases that hydrolyze β-lactam antibiotics, including extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases.

These enzymes are often located on mobile genetic elements, facilitating rapid dissemination of resistance among bacterial populations [36]. Carbapenemase production is another major mechanism, leading to carbapenem-resistant *K. pneumoniae* (CRKP) and reduced carbapenem susceptibility [37].

As noted in the present study (Figure 2), many isolates that exhibited high resistance to β-lactam antibiotics lacked detectable β-lactamase genes. This suggests alternative mechanisms such as capsule formation, biofilm production, porin modification, or alterations in penicillin-binding proteins, as reported in several studies [8, 38, 39].

In Iraq, a notable rise in multidrug-resistant strains has been observed. High prevalence of β-lactam resistance, particularly to penicillins and cephalosporins, is largely driven by ESβL genes such as *blaSHV, blaTEM,* and *blaCTX-M* [40]. Alarmingly, carbapenem resistance is increasing, mediated primarily by carbapenemase genes and New Delhi metallo-β-lactamase (NDM) [16, 41, 42]. In contrast, many international reports attribute β-lactam resistance mainly to *blaTEM* and *blaSHV* [35, 43, 44]. The *ctx-M1* gene plays a critical role in extended-spectrum cephalosporin resistance, with local increases likely reflecting selective pressure from the extensive use of third-generation cephalosporins such as ceftriaxone and cefotaxime in routine clinical practice [45, 46]. Cephalosporins and monobactams have historically been widely used to treat Gram-negative infections, including those caused by *K. pneumoniae*. However, in recent years, their effectiveness has declined due to the emergence of ESβL-and carbapenemase-producing isolates, contributing to a global rise in resistance [36, 47-51].

Aminoglycoside resistance in *K. pneumoniae* is primarily driven by aminoglycoside-modifying enzymes, including acetyltransferases, nucleotidyltransferases, and phosphotransferases. These enzymes inactivate aminoglycosides through acetylation, adenylation, or phosphorylation, effectively neutralizing the antibiotic [37]. The genetic determinants for these enzymes are often located on transferable elements such as plasmids and transposons, enabling their spread among bacterial populations. Additionally, 16S rRNA methyltransferases (e.g., ArmA and RmtB) confer high-level resistance by methylating the ribosomal target, therby preventing aminoglycoside binding [19].

According to the present findings, the widespread presence of *aph(3')-Ia, aac(3')-IIa*, and *aac(6')-Ib* suggests that aminoglycoside resistance is primarily associated with these modifying enzymes [30, 52]. The high rates of aminoglycoside-resistant isolates may reflect the extensive use of these antibiotics in Iraqi clinical settings, which has likely contributed to the emergence of resistant strains [53, 54]. In recent years, resistance to aminoglycosides-particularly when combined with carbapenem resistance-has been increasingly reported worldwide [55, 56].

5. Conclusions

This study highlights the high prevalence of multidrug resistance among *K. pneumoniae* isolates in Najaf, Iraq. The predominance of *blaTEM, blaSHV,* and aminoglycoside-modifying enzyme genes underscores the urgent need for continuous surveillance and rational antibiotic use. Resistance was found to correlate with both genotypic and phenotypic traits, but alternative mechanisms such as capsule production and biofilm formation may also contribute.

Effective infection control strategies, combined with molecular monitoring of resistance determinants, are essential to limit the spread of multidrug-resistant *K. pneumoniae* in healthcare settings.

5.1 Limitation of the Study

Limited specimens' size and number of isolates may provide little information about the sources of infection. This study was conducted only in Najaf province, Iraq, so the findings may not be representative of the entire country or the broader region.

Author Contributions

Investigation, methodology, resources, and experimental validation: Heba Salman Mahdi Alabayji; supervision, data curation, formal analysis, project administration: Ahlam Kadhim Naeem; software, manuscript drafting: Heba Salman Mahdi Alabayji; manuscript review and editing: Ahlam Kadhim Naeem, Heba Salman Mahdi Alabayji. All authors reviewed and approved the final manuscript.

Funding

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Competing Interests

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

AI-Assisted Technologies Statement

ChatGPT was used to improve the English language of the text in terms of grammar and spelling, as well as and plagiarism reduction. All scientific content, data interpretation, and conclusions were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the content of the manuscript.

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