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Genomic Characterization of the aadA Aminoglycoside Resistance Gene in Multidrug-Resistant Klebsiella pneumoniae Isolated from Urinary Tract Infections Using Whole-Genome Sequencing

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and are increasingly associated with multidrug-resistant (MDR) Gram-negative bacteria, particularly *Klebsiella pneumoniae*. This study aimed to isolate and characterize bacterial pathogens from urine samples of female patients clinically suspected of having UTIs and to investigate sequence variations in the *aadA* aminoglycoside resistance gene of MDR *Klebsiella pneumoniae* using whole-genome sequencing (WGS). A total of 200 midstream urine samples were collected and examined using culture, microscopy, biochemical tests, and the VITEK 2 automated identification system. Among the recovered bacterial isolates, *Escherichia coli* was the predominant pathogen, while *K. pneumoniae* represented 22 isolates (17.9%) of the identified urinary pathogens. Among the recovered bacterial isolates, Escherichia coli was the predominant pathogen, while *K. pneumoniae* represented 22 isolates (17.9%) of the identified urinary pathogens. Among the 22 *K. pneumoniae* isolates, one isolate (4.5%) was classified as multidrug-resistant according to CLSI-based antimicrobial susceptibility interpretation. This confirmed MDR *K. pneumoniae* isolate was selected for WGS analysis to characterize aadA-associated genetic variations. The confirmed MDR *K. pneumoniae* isolate was selected for WGS analysis to characterize aadA-associated genetic variations. Sequencing analysis revealed approximately 70 sequence variants within the aadA gene region, including missense, synonymous, upstream, and downstream variants. Several coding-region substitutions, including p.Ile6Thr, p.Val7Ile, and p.Pro9Leu, were detected, suggesting possible structural or functional effects on the encoded aminoglycoside adenylyltransferase enzyme. These findings indicate considerable genetic variability within the aadA resistance gene and highlight the usefulness of WGS for molecular characterization and surveillance of antimicrobial resistance in clinically important *K. pneumoniae* isolates.

Keywords: Urinary tract infections, *Klebsiella pneumoniae*, Multidrug resistance, Whole genome sequencing, aadA gene, Antimicrobial resistance.

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Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a major healthcare problem due to their high prevalence and increasing antimicrobial resistance rates. Gram-negative bacteria are considered the primary causative agents of UTIs, particularly Escherichia coli UTI and *Klebsiella pneumoniae* infection[1]. Recently, *K. pneumoniae* An important opportunistic pathogen due to its ability to acquire multiple antimicrobial resistances (meaning different forms of the same infection are resistant), creating MDR (multidrug-resistance) strains that make treatment more difficult and result in greater severity of infection[2].

Klebsiella pneumoniae has several virulence factors that contribute to its pathogenicity, including the development of capsules, biofilms, adhesins and the mechanisms by which *K. pneumoniae* acquires iron through its use of siderophores; these virulence factors provide *K. pneumoniae* with a better chance of surviving and persisting in the host environment [3]. In addition, one way K. pneumoniae develops resistance to aminoglycosides is through the production of aminoglycoside-modifying enzymes, which are genes associated with the development of resistance to aminoglycosides such as the aadA gene[4]. These genes provide a mechanism by which the bacterial cell becomes resistant due to the inactivation of aminoglycosides through the production of aminoglycoside-modifying enzymes.

Globally, the epidemiology of multidrug-resistant Klebsiella pneumoniae has become increasingly alarming. Recent data from the WHO Global Antimicrobial Resistance Surveillance Report indicated that antimicrobial resistance data were collected from more than 23 million bacteriologically confirmed infections across 104 countries in 2023, including bloodstream infections, urinary tract infections, gastrointestinal infections, and urogenital infections. More importantly, over 55% of *K. pneumoniae* isolates worldwide are now resistant to third-generation cephalosporins, while resistance to carbapenems and fluoroquinolones is also increasing, thereby narrowing available therapeutic options. In the WHO Eastern Mediterranean Region, available surveillance data remain limited, which may contribute to underestimation of resistant and hypervirulent *K. pneumoniae* strains. In Iraq, recent local studies have reported high resistance levels among *K. pneumoniae* isolates, including multidrug-resistant phenotypes ranging from 22.7% to 68.9% in clinical isolates, with marked resistance to cephalosporins, β-lactams, sulfonamides, and variable resistance to carbapenems and aminoglycosides. These global, regional, and local findings highlight the clinical importance of MDR K. pneumoniae and support the need for molecular surveillance of resistance determinants such as the aadA gene using whole-genome sequencing [5].

WGS has become a common molecular method being used to find resistance genes, identify variations in the sequence of genes in a genome that indicate an organism's ability to evolve into a multi-drug resistant organism and to understand how bacterial pathogens will

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continue to evolve and develop resistance to antimicrobials. Sequence analysis of genes associated with resistance can yield important knowledge regarding how bacteria adapt, how mutations occur, and how genomics of bacteria changes in relation to genes associated with resistance [6].

Although the few local studies regarding the sequence differences in the aminoglycoside resistance genes in multidrug resistant strains of *K. pneumoniae* only focus on the use of whole genome sequencing to determine whether they had one or more copies of the *aadA* resistance gene, they failed to demonstrate any variation between the sequences of the genes obtained from these different strains. Accordingly, this study was intended to achieve two main objectives; namely, to isolate and characterize Gram negative bacteria from patients with urinary tract infections and to identify the presence of sequence variation within the aadA resistance genes of the multidrug resistant *K. pneumoniae* isolated from patients with UTIs, using whole genome sequencing

Materials and Methods

Collection of Clinical Samples

A total of 200 urine samples presumed to be infected with UTIs were collected from various hospitals and medical laboratories. Midstream urine samples were kept in sterile bottles that were brought straight to the laboratory for microbiological examination .

Ethical Considerations

Bacteria used in this study were isolated from clinical UTIs following ethical clearance by the concerned authorities .

Culture Media Preparation

Media suitable for the growth of microorganisms were prepared according to the instructions given by the manufacturers .

Isolation of Gram-Negative Bacteria

An aseptic technique was used to subculture and inoculate bacteria from the urine specimens seven. Flame sterilization was performed between each of the inoculation loop steps to ensure the inoculation loop was free of germs. The inoculated plates containing the bacteria were incubated in an aerobic environment at 37 °C for a period of 24 hours. Bacterial colonies that were grown on the MacConkey agar incompletely cultured would be classified as either lactose fermenting or non-lactose fermenting based on their appearance characteristics as they relate to each other. The non-lactose fermenting bacterial colonies were further subculturized onto Eosin Methylene Blue (EMB) agar. The EMB agar plates were incubated at 37°C for a period of 24 hours. The morphological characteristics and the biochemical properties of the bacterial colonies

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that were cultured would provide identification of what organism the bacterial colony(s) is (are) present..

Identification of Uropathogenic Gram-Negative Bacteria

Morphology

The morphological characteristics of bacterial colonies in selective media. Cell morphology (shape, size, arrangement, staining behavior and spore formation) was assessed using Gram staining and microscopic inspection for their detail at low magnification[8].

Biochemical Testing

Biochemical tests (Indole, Voges–Proskauer, Citrate Utilization, Triple Sugar Iron, Oxidase, and Catalase test) were conducted for the identification of bacterial isolates, then identification was confirmed Using VITEK 2 System. Bacteria were identified with the VITEK 2 system via 64 biochemical tests and 15 antibiotic testing methods. As per the manufacturer's instructions, the system was used for characterization of bacterial isolates[12].

Maintenance of Uropathogenic Gram-Negative Bacterial Isolates

Bacteria isolates were preserved as reported by [13]

A. Short-Term Storage:Bacteria isolates were then kept for multiple days on MacConkey agar plates sealed with Parafilm in a refrigerator at 4°C .

B. Medium-Term Storag: Bacteria isolates were stored in medium term in brain heart infusion (BHI) slants in tightly capped universal tubes containing 5–8 ml of medium at 4°C .

C. Long-Term Storage: A colony of bacteria was inoculated in BHI broth and incubated for 24 hours at 37°C. To maintain these culture volumes, a mixture of 1.5 ml glycerol and 8.5 ml bacterial suspension was stored at -20°C.

Antimicrobial susceptibility testing of confirmed *K. pneumoniae* isolates was performed using the VITEK 2 automated system according to the manufacturer’s instructions. The susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Isolates were classified as multidrug-resistant (MDR) when they showed non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. The MDR *K. pneumoniae* isolate that fulfilled this criterion was selected for whole-genome sequencing analysis.

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Genomic DNA Isolation

Bacterial genomic DNA isolation was conducted using commercially available genomic DNA extraction kits according to the manufacturer's protocol from newly acquired bacterial colonies. Cultures of bacteria grown overnight on MacConkey agar plates were put in phosphate-buffered saline (PBS) buffer. Following the protocol from the kit, bacterial cells were lysed, protein digestion, and DNA purification was performed. The amount and purity of genomic DNA was determined using NanoDrop spectrophotometry using A260/A280 absorbance ratio. Isolated DNA was held at −20°C for future WGS study .

Whole Genome Sequencing

Whole genome sequencing libraries were prepared from purified genomic DNA and sequenced using the Illumina paired-end sequencing platform with a read length of 151 bp. Raw sequencing reads underwent quality control analysis using FastQC software (v0.11.7), while adapter trimming and low-quality read filtering were performed using Trimmomatic software (v0.38).

Filtered reads were aligned against the reference genome of *Klebsiella pneumoniae* using the BWA-MEM algorithm (v0.7.17), and duplicate reads were removed using Picard tools. Variant calling for SNPs and small insertions/deletions (InDels) was performed using GATK software (v4.2.0.0), while variant annotation and prediction of amino acid changes were conducted using SnpEff software (v5.0e).

Results and Discussion

Sample collection

In the present study, a total of 200 midstream urine samples were collected from female patients clinically suspected of having urinary tract infections (UTIs) at Al-Fallujah Teaching Hospital in Al-Anbar Governorate between October and December 2025. The mean age of the participants was 35 ± 5 years. After microbiological examination, 150 out of 200 urine samples yielded significant bacterial growth, while the remaining samples showed no significant growth and were excluded from further bacterial characterization.

Urine samples were cultured on MacConkey agar for the isolation of Gram-negative bacteria associated with UTIs. Following incubation at 37°C for 24 hours, 150 cultured urine samples yielded bacterial growth and were subsequently subjected to morphological, microscopic, biochemical, and automated identification.

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Morphological and Microscopic Characterization

Positive urine cultures were further examined for morphological and microscopic characterization. Colonies grown on MacConkey agar were evaluated according to their size, color, margin, surface appearance, lactose fermentation pattern, and mucoid consistency. Representative colonies were then subjected to Gram staining to confirm bacterial cell morphology and Gram reaction before proceeding to biochemical and automated identification.

As illustrated in Figure (1), suspected *E. coli* UTI colonies appeared as smooth, circular pink to dark-red colonies on MacConkey agar. The pink coloration resulted from lactose fermentation by Escherichia coli, which produced acidic metabolites leading to a reduction in medium pH and subsequent activation of the neutral red indicator.

Figure (1): *Escherichia coli* bacteria cultured on MacConkey agar

Variation in colony color intensity was observed among different isolates, ranging from light pink to deep red, reflecting differences in lactose fermentation activity and acid production rates among bacterial strains. These observations confirmed the strong lactose-fermenting capability of *E. coli* isolates.

The obtained findings were consistent with previous studies reporting that lactose-fermenting Enterobacteriaceae, including *E. coli* and *Klebsiella* spp., produce pink colonies on MacConkey agar, whereas non-lactose fermenters such as *Proteus*, *Salmonella*, and *Pseudomonas* spp. produce pale or colorless colonies due to the absence of lactose fermentation.

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For additional confirmation, representative lactose-fermenting colonies were subcultured onto Eosin Methylene Blue (EMB) agar. As shown in Figure (2), *E. coli* isolates demonstrated a characteristic green metallic sheen with a dark central zone, representing one of the most distinctive confirmatory morphological characteristics of *E. coli*.

Figure (2): *Escherichia coli* bacteria cultured on EMB agar

The formation of the metallic sheen was attributed to vigorous lactose fermentation and excessive acid production, resulting in precipitation of eosin and methylene blue dyes on the bacterial colony surface.

In addition to *E. coli*, several isolates exhibited morphological characteristics consistent with *K. pneumoniae* infection. As demonstrated in Figure (3), *Klebsiella pneumoniae* isolates appeared as large, round, highly mucoid, glistening pink colonies on MacConkey agar.

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Figure (3): Klebsiella pneumoniae bacteria cultured on MacConkey agar

The mucoid appearance of the colonies was attributed to the presence of a thick polysaccharide capsule, which is considered one of the major virulence determinants of *K. pneumoniae*. This capsule enhances bacterial survival by protecting against phagocytosis and promoting persistence within the host.

Microscopic examination using Gram staining further confirmed the cultural findings. The bacterial isolates appeared as Gram-negative pink-stained rods under light microscopy. The cells were short to medium in length, slender, non-spore forming, and arranged singly or in pairs, consistent with the morphological characteristics of Gram-negative uropathogens.

Overall, the combined cultural and microscopic findings confirmed the successful isolation and identification of Gram-negative bacterial pathogens associated with urinary tract infections, particularly *E. coli* and *K. pneumoniae*.

The present study demonstrated that MacConkey agar and EMB agar remain highly reliable selective and differential media for the preliminary identification of Gram-negative uropathogens. The predominance of lactose-fermenting *E. coli* isolates producing pink colonies on MacConkey agar and metallic sheen colonies on EMB agar is consistent with the classical microbiological characteristics of UPEC reported in previous studies.

The characteristic metallic sheen observed on EMB agar reflects the strong lactose fermentation ability of *E. coli*, resulting in rapid acid production and dye precipitation. Similar findings have been reported [14] who described the green metallic sheen as a diagnostic hallmark for *E. coli* identification.

The identification of highly mucoid *K. pneumoniae* colonies is of particular clinical importance because the mucoid phenotype is directly associated with capsular polysaccharide

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production, which represents one of the major virulence determinants of the organism. Capsule formation enhances bacterial resistance to host immune defense mechanisms, including phagocytosis and complement-mediated killing. These findings agree with previous reports describing *K. pneumoniae* as an opportunistic pathogen capable of causing severe urinary tract infections and nosocomial infections.

Microscopic examination further supported the cultural identification findings, as Gram staining demonstrated typical Gram-negative rod-shaped bacterial morphology. The agreement between microscopic and cultural findings confirms the reliability and accuracy of the diagnostic procedures used in this investigation.

Biochemical characterization was performed for approximately 150 bacterial isolates that demonstrated growth on MacConkey and EMB agar and exhibited morphological characteristics consistent with Gram-negative bacteria. The applied biochemical tests included indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, oxidase, and catalase assays for accurate differentiation of bacterial isolates associated with UTI, Table (1).

Table (1): Results of biochemical tests for bacteria isolated from urinary tract infections

Bacterial isolate		**Indole**	**Methyl Red**	**Voges–Proskauer**	**Citrate**	**Oxidase**	**Catalase**
Uropathogenic *Escherichia coli*		+	+	−	−	−	+
Klebsiella spp.		−	−	+	+	−	+
Enterococci		−	−	−	−	−	−
Proteus spp.		−	+	−	+	−	+

The biochemical profile of UPEC UTI isolates showed positive reactions for indole, methyl red, and catalase tests, whereas negative reactions were observed for Voges–Proskauer, citrate utilization, and oxidase tests. This profile confirmed the ability of *E. coli* isolates to produce indole and perform mixed-acid fermentation.

In contrast, *K. pneumoniae* infection isolates demonstrated negative indole and methyl red reactions but positive Voges–Proskauer, citrate utilization, and catalase reactions. The positive citrate utilization test confirmed the ability of Klebsiella isolates to utilize citrate as a sole carbon source, while the positive Voges–Proskauer reaction indicated acetoin production during glucose fermentation.

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Proteus isolates exhibited positive methyl red, citrate, and catalase reactions, while indole, Voges–Proskauer, and oxidase tests were negative. These biochemical characteristics supported differentiation of *Proteus* spp. from other Gram-negative bacterial isolates.

The isolates identified as *Enterococcus* spp. showed negative reactions for all tested biochemical parameters included in this study. Although enterococci are Gram-positive cocci rather than Gram-negative bacilli, they were recovered during routine urinary culture screening.

Overall, the combination of cultural appearance, Gram staining, and biochemical characterization provided reliable identification and differentiation of the major bacterial pathogens associated with urinary tract infections.

For additional confirmation, selected isolates were further analyzed using the VITEK 2 automated identification system, which demonstrated high accuracy in species-level bacterial identification, table (2).

Table (2): Types and frequency of bacterial isolates recovered from urinary cultures

No.	Bacterium	Number (%)
1	*Escherichia coli*	83 (67.4%)
2	*Klebsiella pneumoniae*	22 (17.9%)
3	*Enterococcus faecalis*	12 (9.8%)
4	*Proteus mirabilis*	6 (4.9%)
Total		123 (100%)

The VITEK 2 identification results demonstrated that *E. coli* represented the predominant bacterial isolate, accounting for 67.4% of all recovered pathogens. *K. pneumoniae* represented the second most common isolate (17.9%), followed by *E. faecalis* (9.8%) and *P. mirabilis* (4.9%).

The predominance of *E. coli* strongly supports its established role as the principal etiological agent of UTI. In comparison, the recovery of *K. pneumoniae* remains clinically important because of its virulence properties and association with multidrug-resistant phenotypes.

The obtained biochemical results were consistent with the standard identification profiles previously reported for major uropathogenic bacteria. The biochemical pattern observed for UPEC agreed with findings according to [15] who described *E. coli* as indole positive, methyl red positive, citrate negative, and Voges–Proskauer negative.

Similarly, the biochemical characteristics of *K. pneumoniae* isolates observed in the present study were consistent with previous reports [16], which identified *K. pneumoniae* as citrate positive and Voges–Proskauer positive while remaining indole negative.

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The predominance of *E. coli* among urinary isolates was also in agreement with global and regional epidemiological studies indicating that UPEC remains the major causative pathogen of UTI. In addition, the detection of *K. pneumoniae* isolates is clinically significant because this pathogen is frequently associated with multidrug resistance and nosocomial UTI [17].

Overall, the combination of biochemical testing and automated VITEK 2 identification provided reliable characterization of urinary bacterial isolates and supported accurate differentiation of clinically important uropathogens.

Whole Genome Sequencing Analysis of the aadA Resistance Gene in Multidrug-Resistant *Klebsiella pneumoniae*

Whole genome sequencing (WGS) analysis was performed for the selected multidrug-resistant (MDR) Multidrug-resistant *K. pneumoniae* isolate in order to investigate the genetic determinants associated with antimicrobial resistance. Sequence variant analysis identified multiple nucleotide alterations within the aadA resistance gene, which is known to encode aminoglycoside adenylyltransferase enzymes involved in resistance to aminoglycoside antibiotics.

The sequencing data revealed approximately 70 sequence variants distributed across the aadA gene region. Different mutation types were detected, including missense variants, synonymous variants, upstream gene variants, and downstream gene variants. Among these alterations, missense mutations represented the most biologically significant category because they resulted in amino acid substitutions within the encoded protein sequence, potentially affecting protein structure and resistance activity.

Several amino acid substitutions were identified within the *aadA* coding region, including p.Ile6Thr, p.Val7Ile, p.Pro9Leu, and additional coding sequence changes. These mutations indicate active genetic variability within the resistance-associated region of the MDR isolate, Table (4).

Table (4): Major sequence variations identified in the *aadA* resistance gene of MDR *Klebsiella pneumoniae*

Genomic position	Nucleotide change	Variant type	Amino acid change
19526	T>C	Missense variant	p.Ile6Thr
19528	G>A	Missense variant	p.Val7Ile
19529	C>T	Missense variant	p.Pro9Leu

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19534	G>A	Missense variant	Amino acid substitution
19536	A>T	Synonymous variant	No amino acid change
19541	C>A	Missense variant	Amino acid substitution

The detected missense mutations suggest possible structural and functional modifications in the aminoglycoside resistance protein encoded by the *aadA*. Since aminoglycoside-modifying enzymes are directly involved in antibiotic inactivation, these sequence alterations may contribute to the enhanced multidrug-resistant phenotype observed in the investigated isolate.

In addition to coding-region mutations, multiple upstream and downstream variants were also identified surrounding the *aadA* locus. These regulatory-region variations may influence gene expression efficiency, transcriptional regulation, or promoter activity, potentially affecting resistance gene expression levels Table (5).

Table (5): Distribution of variant types identified in the *aadA* gene region

Variant category	Biological significance
Missense variants	Amino acid substitutions affecting protein structure
Synonymous variants	Silent mutations without amino acid changes
Upstream gene variants	Possible effects on promoter and transcription regulation
Downstream gene variants	Potential influence on gene stability and regulation

The presence of extensive sequence variability within the *aadA* resistance gene strongly supports its association with the MDR phenotype in *K. pneumoniae*. The *aadA* gene belongs to a well-characterized family of aminoglycoside resistance genes that encode aminoglycoside adenylyltransferases capable of modifying and inactivating aminoglycoside antibiotics. Therefore, mutations detected within this gene may enhance bacterial adaptation and antimicrobial resistance efficiency.

Furthermore, the identification of numerous mutations within a clinically important resistance gene highlights the genomic plasticity of MDR *K. pneumoniae* isolates and emphasizes the importance of whole genome sequencing for understanding the molecular mechanisms underlying antimicrobial resistance. These findings provide valuable insights into the genetic basis of MDR development and support the role of WGS as a powerful tool for resistance surveillance and molecular characterization of clinically significant pathogens.

The present study used whole genome sequencing to investigate sequence variations within the *aadA* resistance gene in multidrug-resistant (MDR) *K. pneumoniae* infection. The

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identification of approximately 70 sequence variants within the *aadA* gene region indicates considerable genetic variability in this resistance-associated locus. *K. pneumoniae* is recognized as an important opportunistic pathogen with a remarkable ability to acquire antimicrobial resistance determinants, particularly in hospital-associated and urinary tract infections. According to Effah and colleagues (2020), the increasing emergence of MDR *K. pneumoniae* represents a major global public health threat because of limited treatment options and rapid dissemination of resistance genes [18].

The aadA gene encodes aminoglycoside adenylyltransferase enzymes responsible for enzymatic modification and inactivation of aminoglycoside antibiotics. Aminoglycoside-modifying enzymes are among the most important mechanisms mediating aminoglycoside resistance in Gram-negative bacteria [19]. Therefore, the detection of multiple sequence alterations within the *aadA* gene in the present MDR isolate strongly suggests a possible association between these mutations and antimicrobial resistance development.

Among the detected sequence alterations, missense variants represented the most biologically important category because they resulted in amino acid substitutions within the encoded protein sequence. Several substitutions, including p.Ile6Thr, p.Val7Ile, and p.Pro9Leu, were identified in the coding region of the *aadA* gene. These amino acid changes may affect protein conformation, enzymatic activity, or substrate specificity. demonstrated that structural alterations in aminoglycoside adenylyltransferase enzymes can influence resistance efficiency and antibiotic modification activity [20].

In addition to missense mutations, synonymous, upstream, and downstream variants were also detected surrounding the *aadA* locus. Although synonymous mutations do not alter amino acid sequences directly, they may still reflect genomic evolution and adaptation. Furthermore, upstream and downstream variants may influence promoter activity, transcriptional regulation, and gene expression levels emphasized that whole genome sequencing enables the detection of both coding and regulatory variants associated with bacterial antimicrobial resistance mechanisms [21].

The extensive sequence variability identified in the present study also reflects the high genomic plasticity of MDR *K. pneumoniae*, have possesses a highly dynamic genome and can rapidly acquire resistance genes through plasmids, transposons, integrons, and horizontal gene transfer mechanisms. Continuous exposure to antimicrobial selective pressure may therefore contribute to the accumulation of genomic mutations and emergence of resistant bacterial clones [22].

The present findings are consistent with [23], who identified aminoglycoside resistance-associated genes among MDR *K. pneumoniae* isolates and highlighted their role in resistance

dissemination. Similarly, demonstrated the usefulness of whole genome sequencing for identifying resistance-associated mutations and understanding genomic variation patterns in MDR *K. pneumoniae* clinical isolates.

Overall, the detected sequence variations within the *aadA* resistance gene support its potential association with aminoglycoside resistance and MDR development in the investigated *K. pneumoniae* isolate. However, further molecular and functional analyses are still required to confirm the direct biological effects of these mutations on protein activity and antimicrobial resistance phenotypes.

Conclusions

The present study confirmed the predominance of Gram-negative bacteria among urinary tract infection isolates, with Klebsiella pneumoniae representing an important uropathogen associated with multidrug-resistant phenotypes. Whole-genome sequencing analysis revealed multiple sequence variations within the aadA aminoglycoside resistance gene, including missense, synonymous, upstream, and downstream variants that may contribute to aminoglycoside resistance and the development of multidrug resistance.

These findings emphasize the importance of whole-genome sequencing as a powerful molecular tool for the characterization, monitoring, and surveillance of antimicrobial resistance in clinically significant bacterial pathogens. Furthermore, the detected genetic variations may provide valuable insights into the molecular mechanisms underlying resistance evolution in MDR Klebsiella pneumoniae. However, further functional and phenotypic studies are required to confirm the direct effects of these variants on antimicrobial resistance.

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Author's Declaration

We hereby confirm that all the tables and data presented in this manuscript are original and have been prepared by the authors.

This study was conducted as an exploratory cross -sectional investigation at Al-Fallujah Teaching Hospital between October and December 2025. The study protocol was approved by the Institutional Ethics Committee (Approval No. 20250113, dated 26/10/2025). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki (World Medical Association, 2013). This approval reflects our commitment to ethical research standards and the protection of participants' rights and well-being.

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