

Comprehensive Genomic Characterization of a Drug-Resistant *Klebsiella pneumoniae* Clinical Isolate in Iraq Using Whole Genome Sequencing

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What's Known

- The next-generation technique is well known for whole-genome sequencing to identify bacterial genomic characteristics, including antibiotic resistance genes. Resistance genes were mentioned in previous studies but not identified in Iraq.

What's New

- This is considered the first study to know the bacterial whole genetic sequence in Iraq, rather than being the first study to draw the complete genetic map of this bacterium. Moreover, it is the only study in Iraq to identify prophage and CRISPR-Cas system, insertion sites, and bacterial serotype in the genetic sequence, and finally, the first to draw the genetic tree comparing our isolate to other countries.

Abstract

Background: *Klebsiella pneumoniae* is a Gram-negative encapsulated opportunistic pathogen, which presents a major threat to public health due to its ability for multi-antibiotic drug resistance. It is responsible for 30% of Gram-negative bacterial infections, including nosocomial infections, pneumonia, septicemia, and urinary tract infections. The study aimed to analyze the key phenotypic and genetic features of clinical *K. pneumoniae* isolates.

Methods: Between 2022 and 2023, a total of 91 strains of *Klebsiella pneumoniae* were collected from Al-Imamian Al-Kadhimiyain Medical City (IKMC) and characterized using the VITEK-2 technique. Whole-genome sequencing (WGS) was employed to characterize the extreme drug-resistant strain. The whole genome was extracted and sequenced using the Next Generation Sequencing (NGS) technique. The genome of our bacterial isolate was analyzed using different bioinformatics tools such as Galaxy workflow, SPAdes, PROKKA, and Staramr.

Results: The analysis identified *Klebsiella pneumoniae* serotype K36:O2a and sequencing type ST-437, containing 15 different plasmids carrying 54 resistance genes and more than 100 virulence genes with one region of CRISPR and no *Cas*. The sample obtained four intact bacteriophages and two questionable ones. Seven insertion sequences were revealed in the analysis as part of Other Mobile Genetic Elements (OMG). Additionally, the 16SrRNA phylogenetic tree identified a higher relationship of the bacteria to the strains from the USA and India than from Iraq.

Conclusion: It is the first study in Iraq to utilize WGS to comprehensively characterize an opportunistic pathogen. The study emphasizes the need for WGS to track the development of resistance and virulence patterns in clinical strains of *K. pneumoniae*.

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Keywords: • Whole genome sequencing • *Klebsiella pneumoniae* • Multidrug efflux pump genes • CRISPR-Cas system • NGS

Introduction

About 30% of Gram-negative infections are caused by *Klebsiella pneumoniae*,¹ a non-motile Gram-negative encapsulated opportunistic bacterium.² which encompasses plentiful

chromosomal and plasmid-encoded antibiotic resistance genes (ARGs). *K. pneumoniae* is one of the leading causes of community-acquired infections, including urinary tract, respiratory, and bloodstream infections.³ Its distribution is a major public health problem, especially with the emergence of multidrug-resistant (MDR) strains.⁴ Another research has demonstrated antibiotic resistance in *K. pneumoniae*, which poses a significant and immediate threat.⁴ Such bacteria have developed resistance to antimicrobial agents by several mechanisms, including alterations in permeability, active efflux, enzymatic modification, degradation, modification of antibiotic targets, acquisition of alternate metabolic pathways, and overproduction of the target enzyme.⁵

This pathogenic microorganism induces infection by various virulence factors, including surface antigens, fimbriae, iron uptake, capsule, serum resistance, outer membrane proteins, toxins, and siderophores, which enable the bacteria to penetrate and proliferate within the host.⁶

Genotyping is essential for identifying this significant pathogen. Multilocus Sequence Typing (MLST) is a molecular technique to determine the genetic association between bacterial isolates. It is mainly used for molecular epidemiological studies of microorganisms that are of public health concern.⁷ MLST analysis has demonstrated that *K. pneumoniae* exhibits a predominantly oligoclonal nature, with a plenty of sequence types (ST) identified, including ST 11, 14, 15, 26, 101, 147, 149, 231, 258, 627, and 977.⁸ Several STs have exhibited geographic specificity, with some becoming both endemic and/or epidemic.⁹

The *K. pneumoniae* capsule is an intricate formation consisting of 16 to 20 various genes, depending on the serotype. Moreover, the capsule functions as a protective barrier for the host's initial inflammatory response by inhibiting opsonization.¹⁰

K. pneumoniae can produce two main porins, OmpK35 and OmpK36. Porins are proteins that enable the movement of hydrophilic substances in both directions across the cell membrane. Due to the lack of outer membrane porins, *K. pneumoniae* exhibits enhanced resistance to cephalosporin and carbapenem, and reduced pathogenicity.¹¹

Whole-genome sequencing (WGS) offers unprecedented resolution and comprehensive information for the entire bacterial genetic material, encompassing all key genomic elements. Beyond bacterial identification and molecular characterization, WGS holds significant potential

for predicting microbial phenotypes.¹²

The objective of the study was to analyze the key phenotypic and genetic features present in the clinical *K. pneumoniae* isolate. It is the first study in Iraq to utilize WGS for a comprehensive characterization of an opportunistic pathogen, thus emphasizing the need to use whole-genomic sequencing to track the development of resistance and virulence patterns in clinical strains of *K. pneumoniae*.

Materials and Methods

Bacterial Isolation and Identification

Ninety-one *K. pneumoniae* isolates were obtained from urine samples collected from Al-Imamian Al-Kadhimiyain Medical City (IKMC) between 2022 and 2023 from children under twelve years old. Primary isolation was performed using MacConkey agar as a selective culture medium. Subsequent identification and confirmation of the isolates were conducted using the VITEK-2 (bioMérieux, Inc., Durham, NC) automated microbial identification system. The strains were subcultured on Luria-Bertani (LB) or DNA extraction and incubated at 37 °C for 24 hours under aerobic conditions.

DNA Extraction

One strain isolated from a 4-day-old infant due to its extreme drug-resistant profile. It was centrifuged at 5000 x g for 10 min to obtain bacterial cultures. Following the manufacturer's procedure for bacterial samples, the DNA was isolated using the QIAamp DNA Mini kit (QIAGEN, Germany). The separated DNA was kept at a temperature of -20 °C until it was time to prepare the library.

Whole-Genome Sequencing (WGS)

Genomic DNA was extracted, followed by WGS on the Illumina HiSeq 2000 platform (Illumina, USA). *De novo* genome assembly was performed using SPAdes (software developers: Russia and USA) (<https://ablab.github.io/spades/>). Draft genomes were annotated using rapid prokaryotic genome annotation (Prokka) (<https://training.galaxyproject.org/training-material/>). The resulting *K. pneumoniae* genome exhibited high data quality and was submitted to the National Center for Biotechnology Information (NCBI).

Data Accessibility

The sequence data have been successfully submitted to the NCBI's database with the BioProject Accession Number PRJNA1130523

(https://www.ncbi.nlm.nih.gov/bioproject?LinkName=biosample_bioproject&from_uid=42208297) and BioSample Accession Number SAMN42208297 (<https://www.ncbi.nlm.nih.gov/biosample/42208297>).

Genome Mapping

The CGView Server (<https://js.cgview.ca/>) was used to produce a comparative circular genome map, with the *Klebsiella pneumoniae* strain HS11286 genome sequence (Accession number: CP003200.1) acting as the reference.

Multilocus Sequence Typing

The following seven housekeeping genes were used to do multilocus sequence typing (MLST): *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*. The Pasteur Institute's BIGS database (<https://bigsd.bpasteur.fr/>) was used to identify allele sequences and STs, reporting only exact allelic profile matches.

Antibiotic Resistance

Bacterial resistance genes and plasmid replicons were identified using the ResFinder tool (<http://genepi.food.dtu.dk/resfinder>) with a 100% identity and an 80% minimum match length.

Virulence Factors

The previously obtained *K. pneumoniae* genome was analyzed using the Virulence Factors Database (<http://www.mgc.ac.cn/VFs/>) to identify known virulence genes. Clusters examined included *fim* (A-H, and K), *mrk* (A-D, and F), *ecp* (A-E), and genes for outer membrane proteins (*ompA*, *ompK35*, *ompK36*), the *virB* cluster (1-11) of the T4SS, and hypermucoviscosity genes *magA* and *rmpA*. Additionally, siderophores and enterobactin genes coded by *fes*, salmochelin coded by *iroN* and *fyuA*, and *iut* clusters, coding for yersiniabactin and aerobactin, respectively, were investigated. Gene queries were based on the CP003200 reference strain, using an identity threshold of 100% and a minimum match length of 80%.

16SrRNA Phylogeny

16S rRNA sequences from our isolate genome sequence were aligned with the NCBI database using Molecular Evolutionary Genetics Analysis (MEGA11) (Pennsylvania State University, USA) (<https://www.megasoftware.net/>). These sequences were used to construct neighbor-joining trees using the MUSCLE tool in MEGA11. The extracted tree was edited using the iTOL server (<https://itol.embl.de/>).

Identification of Mobile Genetic Elements, CRISPR-Cas Region, and Bacterial Serotype

The ISFinder server (<https://isfinder.biotoul.fr/about.php>) was used to detect Other Mobile Genetic Elements by comparing the FASTA to the database. The phage regions were identified and visualized using the PHASTER tool (<https://phaster.ca/>), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) were identified and visualized using the CRISPRFinder tool (<https://crisprcas.i2bc.paris-saclay.fr/CrisprCasFinder/Index>). The Kaptive tool (<https://kaptive-web.erc.monash.edu/jobs>) was employed for capsular typing. It was utilized to identify the K/O capsular type of our isolate. Furthermore, the plasmids were characterized by aligning the contigs of the sample with the plasmid database, and the reads were aligned with the PlasmidFinder database (<http://genepi.food.dtu.dk/resfinder>).

Single Nucleotide Polymorphisms (SNP) and Indel Discovery

Variant Count: The generated WGS data were utilized to identify genetic variations using the NCBI reference genome. Following the elimination of duplicates using Sambamba and the identification of variants using SAMTools (<https://sourceforge.net/p/samtools/mailman/samtools-devel/thread/2F0E69A8-A2DD-4D6E-9EDE-2A9C0506DA0F@sanger.ac.uk/>), data for each variant was collected and categorized based on their respective chromosomes or scaffolds.

Results

The genomic sequences of the bacterial strain were obtained, which were confirmed to be *K. pneumoniae* with serotype K36:O2a and ST437. The genomes had an average assembly length of 2,192,701,200 base pairs, 14,521,200 reads, a GC content of 57.55%, and 7,604 Coding DNA Sequences (CDS).

16SrRNA was analyzed and compared with the NCBI database to conduct the molecular characterization and phylogenetic distance tree of *K. pneumoniae*, revealing that our strain is more genetically related to the USA and India than Iraq (figure 1).

The data analysis revealed the existence of 54 antimicrobial resistance genes that are accountable for the bacterium strain's resistance to different categories of drugs, as shown in table 1. The antibiotic classes identified in our analysis as being resistant are β -lactamases, aminoglycosides, phenicols, macrolides, sulphonamides, quinolones, tetracycline, trimethoprim, Colistin,

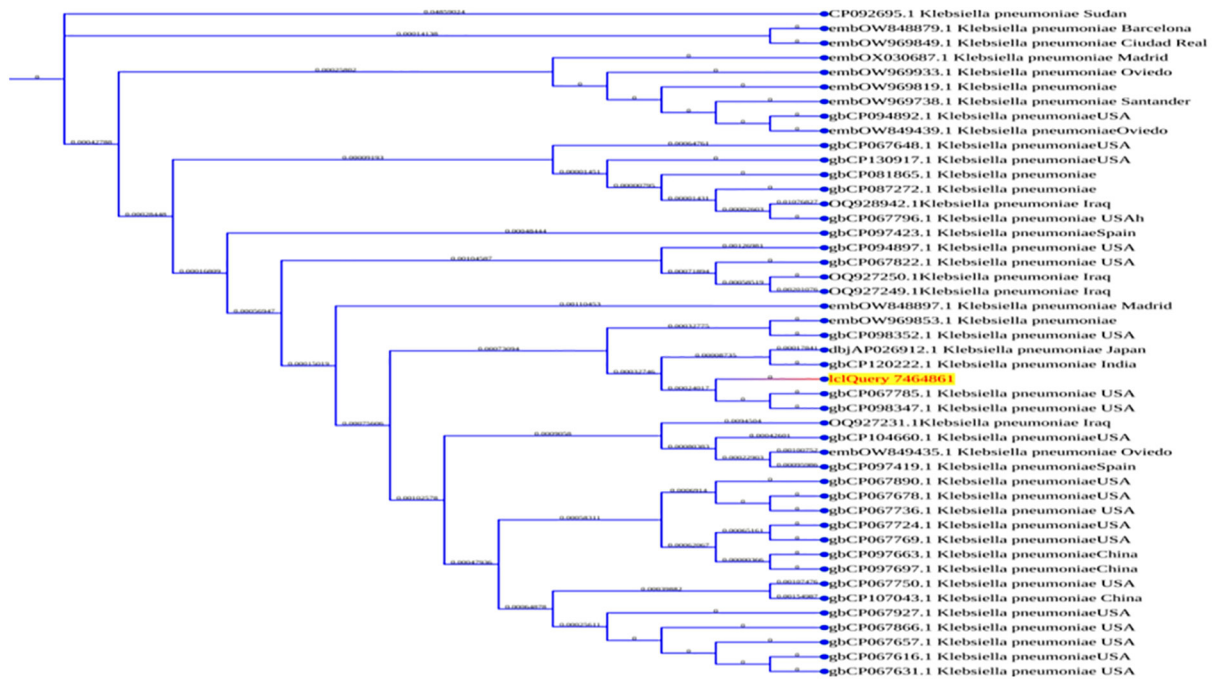


Figure 1: A phylogenetic tree based on nucleotide sequences from the 16S rRNA gene of *Klebsiella pneumoniae* was constructed using MEGA11, with sequence alignment performed through MUSCLE. Bootstrap values, expressed as percentages, were calculated from 1000 resamplings and are displayed at the corresponding nodes in the cladogram.

Table 1: Antibiotic resistance class and resistance genes detected in ST437 *K. pneumoniae* K36:O2a strain

Antibiotic class	Gene detected in this study
Fosfomycin	<i>fosA6</i>
Quinolones	<i>acrA</i> , <i>acrB</i> , <i>acrF</i> , <i>oqxA</i> , <i>oqxB</i> , <i>gyrA</i> , <i>gyrB</i> <i>marA</i> , <i>mdtA</i> , <i>mdtJ</i> , <i>msbA</i> , <i>phoP</i> , <i>phoQ</i> , <i>phoR</i> , <i>pmrD</i> , <i>smvA</i> , <i>soxS</i> , <i>soxR</i>
Phenicol	<i>cat(A)</i> , <i>cat3</i> , <i>catB</i> , <i>catC</i> , <i>catD</i>
Aminoglycosides	<i>aadA2</i> , <i>aph(3')-III</i> , <i>aph(3')-Ia</i> , <i>rmtB</i> , <i>cpxR</i> , <i>cpxA</i> , <i>acrA</i> , <i>acrB</i> , <i>acrZ</i> , <i>acrE</i> , <i>acrF</i> , <i>rpsL</i>
Sulphonamides	<i>sul1</i>
Trimethoprim	<i>dfrA12</i> , <i>dfrA30</i>
Tetracycline	<i>tet(L)</i> , <i>tet(M)</i> , <i>rpsJ</i>
β-lactam	<i>blaCTX-M-15</i> , <i>blaNDM-5</i> , <i>blaOXA-181</i> , <i>blaSHV-182</i> , <i>blaTEM-1B</i> , <i>ampH</i>
Macrolides	<i>ole(D)</i> , <i>erm(B)</i> , <i>erm(T)</i> , <i>mph(A)</i> , <i>msr(C)</i> , <i>msr(A)</i> , <i>msr(B)</i> , <i>emr(C)</i> , <i>car(A)</i> , <i>car(B)</i>
Glycopeptide	<i>Ble</i> , <i>vanA</i> , <i>vanH</i> , <i>vanX</i> , <i>vanY</i>
Aminocoumarin	<i>mdtA</i> , <i>mdtB</i> , <i>mdtC</i> , <i>mdtD</i> , <i>mdtH</i> , <i>mdtK</i> , <i>mdtM</i> , <i>mdtO</i> , <i>mdtL</i> , <i>mdtN</i>

fosfomycin, and glycopeptides. Antibiotic efflux, target replacement, and antibiotic inactivation are three resistance mechanisms caused by the genes shown in [Supplementary table 1](#).

The allele sequences and STs of our *K. pneumoniae* isolate revealed that the strain belongs to the ST437 type; this study is the first to detect this serotype in Iraq. Here, for the first time, we describe a capsular polysaccharide (CPS) cluster for a clinical isolate of *K. pneumoniae* from Iraq. The collected data, including K-serotyping, confirmed the presence of 22 unique CPS genes and 10 unique lipoprotein (LPO) genes (figure 2), which classified our isolate as belonging to the K36:O2a capsular serotype.

The allele sequences and STs of our *K. pneumoniae* isolate revealed that the strain

belongs to the ST437 type. This study is the first to detect this serotype in Iraq.

The analysis of virulence genes indicated the presence of the *mrk* cluster, as shown in [supplementary table 2](#). It supports the idea that type 3 fimbriae are a characteristic feature of *K. pneumoniae* virulence. In addition, the strain harbored a total of fifteen plasmid replicons belonging to the incompatibility types shown in table 2.

The PHAge Search Tool Enhanced Release (PHASTER) was applied to determine whether the bacteriophages were present in the sample. The results of the prophage regions for our sample are shown in figure 3. Our sample included four intact bacteriophages and two questionable ones, which were reported for the first time in Iraq.

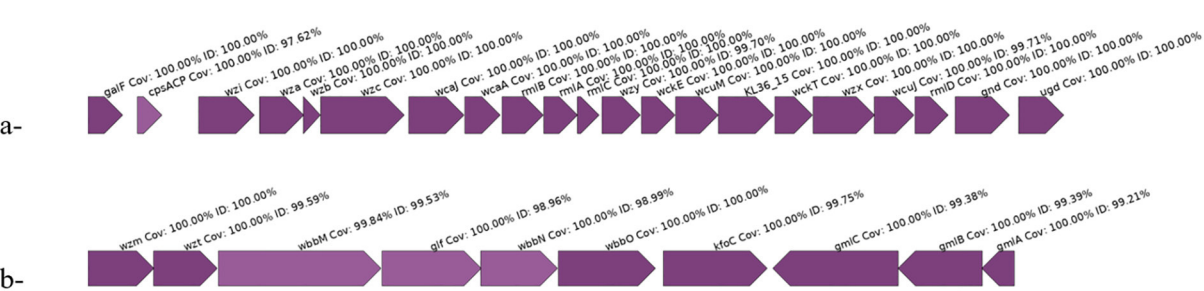


Figure 2: The gene locus of the isolate was analyzed in comparison to the reference genes in the Kaptive database. (a) The K-locus gene arrangement of the *Klebsiella pneumoniae* isolate is displayed, with the gene names indicated above the colored arrows. (b) The O-locus gene arrangement of the *Klebsiella pneumoniae* isolate is also shown, with gene names labeled above the colored arrows. Lighter-colored arrows represent genes with low identity. The colors were fixed by the server

Table 2: Plasmids detected in *K. pneumoniae* clinical isolate. Each plasmid is represented by its coverage and identity to the reference accession number and sequence length

Plasmid	%Identity	%Overlap	HSP Length/Total Length	Start	End	Accession
ColKP3	100	81.79	229/280	2605	2833	JN205800
ColpVC	98.45	100	193/193	310	502	JX133088
IncFIB (K)	100	100	560/560	1723	2282	JN233704
IncFIB (pQil)	100	100	740/740	6822	7561	JN233705
IncFII	100	100	261/261	40165	39905	AY458016
IncFII (K)	100	100	148/148	4355	4502	CP000648
IncX3	100	100	374/374	1638	1265	JN247852
rep14a	100	100	768/768	575	1342	AB038522
rep17	100	100	1041/1041	1679	639	AF507977
rep18a	99.89	100	933/933	2043	1111	AB158402
rep18b	99.43	93.44	527/564	10178	9652	AF408195
rep2	100	100	1494/1494	1961	3454	X92945
repUS15	99.81	100	1041/1041	3330	2290	CP004064
repUS43	100	89.55	1080/1206	1220	141	CP003584
repUS7	100	100	1602/1602	22433	24034	AB206333

HSP: high-scoring segment pairs

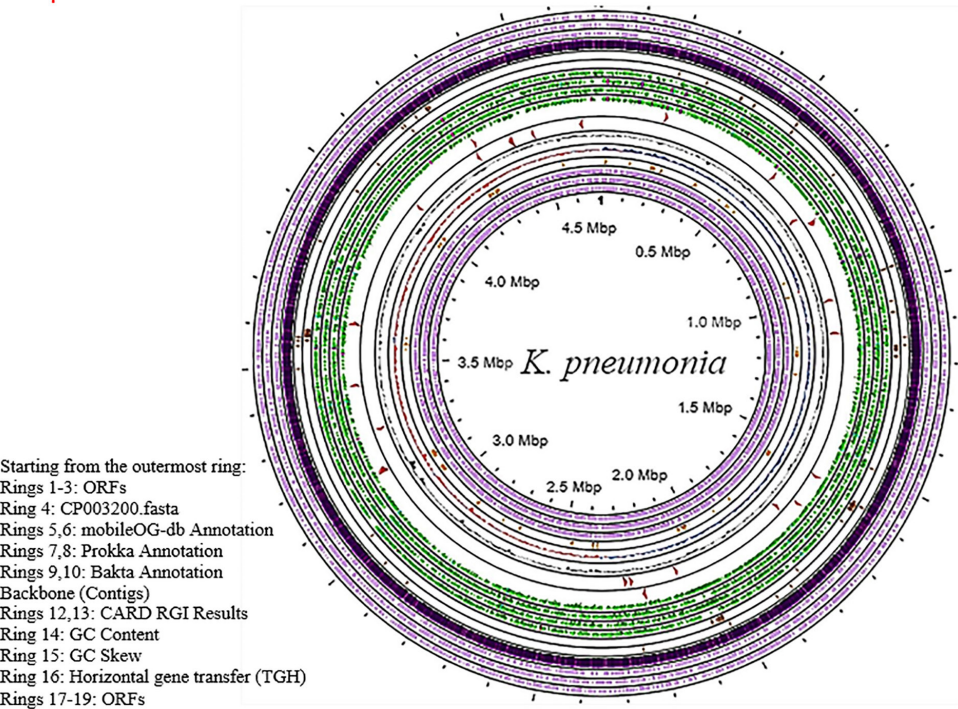


Figure 3: The circular genomic map of our isolate illustrates various features: the black histogram represents the GC content, while the blue-red histogram depicts the GC skew distortion. The reference genome is shown as a dark purple ring, and the coding sequences (CDS) in the isolate are represented by a green ring. The open reading frames (ORFs) are displayed as a light purple ring. Red arrows indicate resistance genes, whereas orange arrows denote horizontal gene transfer (HGT) events.

The intact bacteriophages identified were *Edward_GF*, *Klebsi_ST512_KPC3phi13*, *Salmon_SEN34*, and *Klebsi_ST147_VIM1phi7*. The questionable prophages identified were *Entero_P4* and *Salmon_ST64B*. One CRISPR array was detected in the sample using CRISPRFinder. To our knowledge, this result is the first to detect the CRISPR-Cas system in Iraq. The CRISPRs in the sample were non-Cas-associated, as shown in figure 4. About 10293 Single Nucleotide Polymorphisms (SNPs) were

detected in our isolate, including 6936 transitions and 3357 transversions, 61 insertions, and 71 deletions, causing 3707 protein frameshifts and 909 silent mutations.

Figure 5 shows a circular genomic map of our strain, conducted using a GC viewer. It shows GC content, GC skew, and CDS content in the *K. pneumoniae* K36:O2a ST437 strain. Seven insertion sequences, revealed with the analysis using ISfinder, are shown in table 3 as part of other mobile genetic elements.

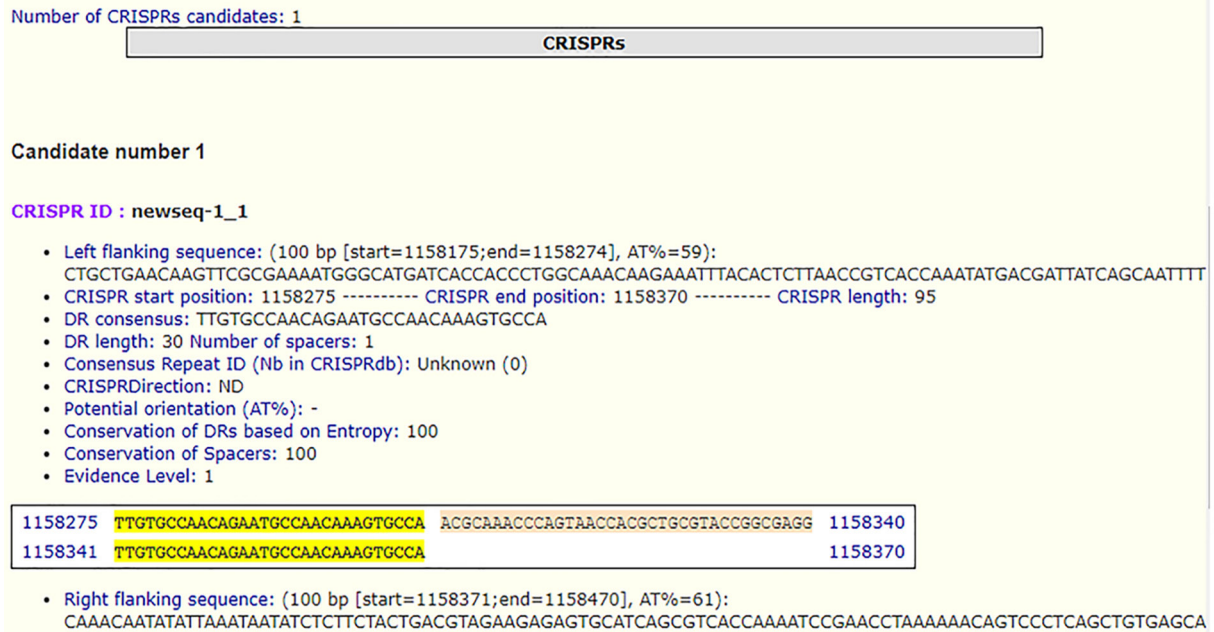


Figure 4: A CRISPR array was identified in the *Klebsiella pneumoniae* K36:O2a isolate, consisting of two direct repeat sequences and one spacer sequence, located between nucleotides 1,158,275 and 1,158,370.

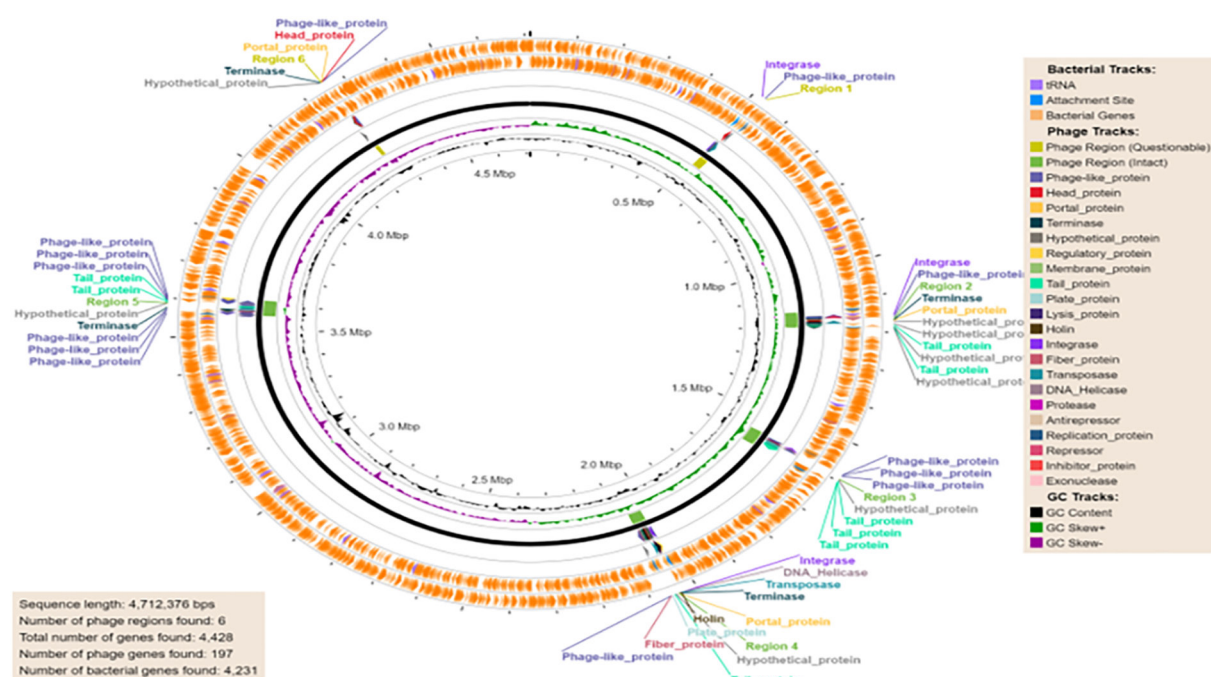


Figure 5: A circular genomic map of the *Klebsiella pneumoniae* isolate was generated using PHASTER, highlighting the regions of phages. The analysis identified a total of 4,428 prophage genes distributed across four intact prophages and two incomplete prophages.

Table 3: Other mobile genetic elements revealing the Insertion Sites (IS) family, group, and E-value for each sequence in the *K. pneumoniae* ST437 strain. This table was concluded using ISfinder

IS Family	Group	Score (bits)	E-value
IS3	IS3	2216	0.0
IS5	IS5	1945	0.0
IS66		1600	0.0
IS3	IS150	1552	0.0
IS5	IS903	963	0.0
IS1		579	2e-161
IS3	IS407	97.6	2e-16

Discussion

The results of this study showed the genetic similarity of our bacterial isolate to strains from the USA and India rather than Iraq. This might be due to various factors, such as global travel¹³ and human migration.¹⁴ Despite a relatively small number of patients, medical tourism has grown in popularity. There have been reports of the ST437 *K. pneumoniae* in North America, Europe, and Asia.¹⁵ In the Indian subcontinent, New Delhi metallo- β -lactamase (NDM)-producing Enterobacteriaceae have been reported to have spread globally.¹⁶ Furthermore, MDR bacteria are a significant clinical concern due to their ability to acquire more 16S rRNA methyltransferase genes, which confer exceptionally high resistance to various aminoglycosides.¹⁷

The WGS of our isolate revealed a large number of resistance genes. This finding contradicts previous reports on other strains of *K. pneumoniae*, including ST152 and ST17, which carried 48 and 36 resistance genes, respectively.¹⁸

The primary resistance mechanism detected is the deactivation of antibiotics through the *aph* genes, which encode aminoglycoside phosphotransferase, and the *aac* genes, encoding aminoglycoside acetyltransferase. The acetyltransferase, *aad* genes, and *ant* genes are responsible for aminoglycoside resistance. This agrees with a study conducted by Ramirez and Tolmasky in 2010.¹⁹ Additionally, nucleotidyltransferase plays a significant role in aminoglycoside resistance.²⁰ The substitution of the antibiotic target is mediated by *sul1*, which encodes a dihydropteroate synthase (DHPS), leading to the bacterial reduced affinity for sulfonamides. The *sul* genes were demonstrated at a high rate, especially the *sul1* gene, when a study conducted in Iran revealed that 71% of *K. pneumoniae* isolates harbored *sul1*.²¹ *dfr* genes are responsible for carrying the trimethoprim resistance *dfrA12* and *dfrA30* detected in our isolate and found in a previous European study.²² The process of rRNA

methylase is utilized in conferring resistance to macrolides through the *erm* genes, which are a widespread mechanism of resistance to the macrolides.²³ Methionine reductase genes (*msr*) were detected in this study. Our findings aligned with the Department of Environmental & Occupational Health Sciences database published by M. C. Roberts (<http://faculty.washington.edu/marilynr/>); the *msr* enzymes are important factors in protecting bacterial species against oxidative stress and in contributing to their pathogenicity.²⁴ To our knowledge, this was the first study to detect *K. pneumoniae's msr* genes in Iraq. Further studies are needed to detect this gene cluster and to understand the necessity of bacteria for those enzyme-coding genes, their regulation, and their precise function in physiology, antibiotic resistance, and bacterial virulence. The *tet* and *oqx*A,B genes present efflux pump ability. The *tet* gene is responsible for conferring tetracycline resistance; a recent investigation on surveillance revealed that around 67% and 45% of *E. coli* and *Klebsiella* species (spp.) in some European nations were resistant to tetracycline.²⁵ The antibiotic resistance frequency was detected in our isolate's genome with 100% identity for the *oqx*A and *oqx*B genes. These genes are responsible for conferring resistance to quinolones. Another researcher observed a significant resistance to this family of antibiotics.²⁶ While *bla* genes are responsible for β -lactamase production our isolate harbors *bla*CTX-M-15, *bla*NDM-5, *bla*OXA-181, *bla*SHV-182, *bla*TEM-1B genes beside *ampH* gene, two enzymes involved in low-level beta-lactam resistance are often chromosomally incorporated in *Klebsiella* spp.; two genes, particularly *TEM-1B*, have been identified as the most commonly found β -lactamase genes in *K. pneumoniae*.²⁷ *fos* genes confer fosfomycin resistance through a pathway involving glutathione transferase; it is expected that these genes were shown to be prevalent as it has been globally documented that fosfomycin is frequently used in combination with aminoglycosides for the treatment of numerous urinary tract infections.²⁸ Furthermore,

antibiotic resistance has been on the rise in Iraq due to the overuse and misuse of antibiotics. In 2021, Al-Taie and colleagues found that 45.8% of individuals were engaged in self-medication of antibiotics without a prescription.²⁹ Clinical samples of *K. pneumoniae* may contribute to the continued ability of this sequence type to evade various classes of antibiotics, as shown by the diverse array of resistance genes and mechanisms identified in this study.¹⁸

The capsular serotypes are known to be strongly related to hypervirulence in *K. pneumoniae*.³⁰ Our results are in line with previous research showing that certain serotypes of *K. pneumoniae* have di-mannose/rhamnose residues, and that genetically modified strains that produce capsules are less able to stimulate polymorphonuclear leucocytes (PMNs).³¹

The strain exhibited both the *ompA* and *ompK35* genes. The higher occurrence of *ompK35* than *ompK36* indicates that *ompK35* plays a crucial function in maintaining the structural integrity of the outer membrane as a porin. On the other hand, the absence of *ompK36* enhances antibiotic resistance, decreases bacterial fitness, and diminishes virulence.³² Specifically, the *blaOXA* carbapenemase gene was found in more than 70% of *K. pneumoniae* strains that lacked the *ompK36* gene, pointing to a possible connection between *ompK36* porin gene deficiency and carbapenem antibiotic resistance.³³ *K. pneumoniae*'s production of siderophores during infection affects the localization of tissues, the infection's spread throughout the body, and the host's survival.³⁴ The presence of the Enterobactin genes in the *K. pneumoniae* genome is expected, as catecholate is commonly encoded in the core genome of *K. pneumoniae*.³

The presence of the IncFIB plasmid, a conjugative plasmid, has been associated with the transmission of several genes in *K. pneumoniae*, including *blaNDM-1*, *blaSHV-12*, *blaCTXM-15*, and *blaOXA-1*.³⁵ *Klebsiella pneumoniae* is the dominant species carrying colistin-resistance-inducing insertion sequence elements on plasmids.³⁶ SKpn1 belongs to the IS3 family and is known for its transposition activity between plasmids and chromosomes of *K. pneumoniae* and *E. coli*.³⁷ Since complete prophages are typically subject to quick deletion from bacteria, it was anticipated that incomplete and questionable prophages would be much more common; instead, our sample obtained four intact bacteriophages and two questionable ones observed for the first time in Iraq. The intact bacteriophages identified were *Edward_GF*, *Klebsi_ST512_KPC3phi13*,

Salmon_SEN34, and *Klebsi_ST147_VIM1phi7*. The questionable prophages identified were *Entero_P4* and *Salmon_ST64B*. This finding contrasts with the study conducted by Kang and colleague in 2023, which reported the lack of *Klebsi_ST512_KPC3phi13* and *Entero_P4* prophages.³⁸ No virulence factors (VF) and antimicrobial resistance (AMR) genes were detected in the prophage sequences found in our isolate. In another study, a large number of VF and AMR genes were found in prophages,³⁸ which showed that plasmids may not significantly contribute to antibiotic resistance in strains, but chromosomally integrated prophages significantly affected strain pathogenicity.

However, the detection of CRISPR sequences has only been found in a small number of *K. pneumoniae*, according to a study by Founou and colleagues in 2019.¹⁸ The Cas proteins associated with CRISPRs play a crucial role in the CRISPR-mediated adaptive immune systems found in bacteria and archaea, particularly the Cas3 helicase.³⁹ In addition, it is regarded as a bacterial defense mechanism as it protects bacteria against phage and horizontal gene transfer by degrading plasmid DNA. On the other hand, a notable discovery of CRISPR-associated Cas in *K. pneumoniae* in highly resistant strains, including ST152 and ST607, indicated the likely involvement of CRISPR-associated Cas3 in gaining resistance genes. This phenomenon can be explained by the existence of CRISPR-associated Cas genes, located between genes that code for proteins involved in metabolism and antibiotic resistance.⁴⁰ Additionally, these two highly resistant strains were discovered to possess numerous phages with the CRISPR-associated *cas* genes, highlighting the need for further research on the development and spread of antibiotic resistance.¹⁸

Only one isolate of *K. pneumoniae* from a single area of Iraq was examined in this study, which can be considered a limitation of this study; larger datasets are required for validation.

Conclusion

The first-ever complete WGS study of the *K. pneumoniae* ST437 strain, which is currently spreading across Iraq, is presented in this research. Our isolates exhibited antibiotic efflux as their primary mode of antimicrobial resistance, with subsequent mechanisms including drug inactivation, protection of antibiotic targets, alteration of antibiotic targets, and replacement of antibiotic targets. Effective management of antimicrobial resistance requires diligent monitoring and surveillance of the prevalence of resistant bacteria, along with identification and

tracking of resistance genes and their specific locations. Implementing these strategies can restrict the occurrence of novel and diverse AMR genes, which have the potential to lead to new human diseases in the future.

Authors' Contribution

S.A: Study concept and design, data acquisition, writing the manuscript; H.A.S: Study concept and design, reviewing the manuscript; K.A: Data analysis and interpretation; O.K: Study concept and design, drafting and reviewing the manuscript. All authors have reviewed, read, and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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