
Molecular typing and genetic diversity of multidrug-resistant *Klebsiella pneumoniae* isolated from Erbil City, Kurdistan Region, Iraq

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ABSTRACT

Background and objective: *Klebsiella pneumoniae*, a Gram-negative encapsulated bacterium, is one of the most significant etiological agents of healthcare- or community-acquired infections worldwide. This study set out to explore the phylogenetic relationships and genetic diversity of multidrug-resistant *K. pneumoniae* using 16S rRNA sequencing and Multilocus Sequence Typing (MLST).

Methods: A total of 298 urine samples were analyzed from patients with symptoms of urinary tract infection. Standard biochemical tests were used for isolate identification. Resistant isolates were further analyzed by molecular diagnosis through 16S rRNA gene sequencing and Multilocus Sequence Typing (MLST), in accordance with international standards.

Results: Out of 298 urine specimens, 49 *K. pneumoniae* isolates were confirmed (isolation rate: 16.4%). Resistance was high: 49% to amoxicillin/clavulanic acid, 38.8% to cefazolin, 34.7% to ceftriaxone, and 12.2% to ertapenem and 16.3% to both imipenem and meropenem among the carbapenems. Multidrug resistance was detected in 65.3% of isolates (32/49), and 18.4% were extensively drug-resistant. MLST testing of 13 MDR strains identified 13 different sequence types, the most dominant (15%) being ST147, followed by ST405 and ST268 (10% each). Both the 16S rRNA sequence analysis and MLST typing showed genetic variation among the resistant isolates, with multiple alleles differing across the seven loci, indicating substantial genetic diversity in the studied isolates.

Conclusion: UTIs associated with antimicrobial-resistant *K. pneumoniae* pose a primary problem in this region. Genetic diversity was found within resistant isolates using molecular typing. Study limitations include the small sample size for MLST (n=13) and the absence of whole genome sequencing, which limit the epidemiological conclusions that can be drawn. Future studies with larger populations and more advanced sequencing methods (such as WGS) are needed for more comprehensive surveillance and a fuller understanding of resistance mechanisms and transmission patterns.

Keywords: *Klebsiella pneumoniae*, Urinary tract infection, 16S rRNA gene sequencing, Phylogenetic analysis, Multilocus sequence typing

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INTRODUCTION

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative, encapsulated bacterium and a major etiological agent of community-associated and healthcare-associated infections worldwide (1). The prevalence of *K. pneumoniae* urinary tract infections (UTIs) is likely to be high throughout Erbil province, Kurdistan Region of Iraq, influenced by factors such as limited availability of high-level diagnostic measures, gaps in infection prevention strategies, and high rates of antibiotic overuse. Molecular characterization of bacterial pathogens has become increasingly important for characterizing *K. pneumoniae* isolates and identifying resistance patterns and the spread of resistant strains (2, 3). These molecular methods offer valuable insight into genetic variability, virulence factors, and resistance mechanisms of *K. pneumoniae*, supporting more effective public health and treatment strategies.

Although molecular surveillance is essential, large-scale molecular typing efforts in Erbil province and beyond remain poorly developed. There is still much to learn about the epidemiology of *K. pneumoniae* in this region, given fragmented surveillance infrastructure, limited research capacity, and economic constraints on the use of advanced molecular technologies.

The 16S ribosomal RNA (rRNA) gene is a highly conserved genetic marker found in the small subunit of the prokaryotic ribosome. It is an important tool in bacterial taxonomy and phylogenetic research, since it is universally distributed and functionally conserved, yet also contains hypervariable regions that allow discrimination at the species and even strain level. The 16S rRNA gene has conserved regions common to all bacteria, along with hypervariable regions (such as V3-V4) that can be used to differentiate

closely related taxa. New high-throughput sequencing techniques have transformed 16S rRNA gene sequencing, with nanopore long-read sequencing of particular interest for the complete identification and analysis of mixed bacterial communities (4).

Phylogenetic trees built from 16S rRNA sequences can reveal genetic relationships, evolutionary history, and possible transmission patterns within a population (5). *K. pneumoniae* identification was confirmed in this study using the 16S rRNA gene, with the additional aim of studying genetic relationships that provide a fuller picture of heterogeneity and strain distribution at the study site.

Although 16S rRNA gene sequencing is useful for bacterial identification and general phylogeny, more elaborate molecular typing methods can offer greater discriminatory power, particularly for epidemiological investigations. Multilocus Sequence Typing (MLST) has become a de facto standard for unambiguous bacterial isolate definition, based on sequences of internal fragments of multiple housekeeping genes. This nucleotide sequence-based method provides high reproducibility and inter-laboratory transferability, making it well suited to global epidemiological surveillance and describing the population structure of bacterial pathogens (6).

The choice of MLST was further supported by its proven effectiveness in *K. pneumoniae* epidemiology. *K. pneumoniae* MLST is a well-established molecular typing technique used to identify high-risk clones and trace their transmission worldwide. The typing scheme presented by Jolley and colleagues at the Institut Pasteur targets seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) under stabilizing selection, which accumulate genetic

variants comparatively slowly and are therefore well suited to evolutionary and epidemiological research (7).

In the present study, the phylogeny, gene relationships, and genetic diversity of multidrug-resistant *K. pneumoniae* isolates from UTIs in Erbil, Iraq, were examined using two approaches: 16S rRNA gene sequencing and MLST. The study design aimed to achieve an in-depth understanding of strain identification, genetic relatedness, and population structure, within the constraints of practicality and budget. The outcomes of this study will help inform understanding of antimicrobial resistance trends and genetic variability of *K. pneumoniae* in the Kurdistan region, forming a basis for future surveillance efforts and local infection control.

METHODS

Sample Collection and Bacterial Identification

A total of 298 urine samples were collected from September to December 2024 at major healthcare facilities in Erbil, Kurdistan Region, Iraq, in this cross-sectional study of patients with clinical signs of UTI. A convenience sampling method was used, based on patients presenting to the participating hospitals during the study period. Specimens were processed as soon as possible after collection, using aseptic transport to prevent bacterial lysis and preserve sample integrity. Although the study used an adequate overall sample size, one limitation is that only a subset of samples could undergo molecular typing, since this requires limited resources and access to advanced sequencing facilities in the area.

The sample size used in this study has some limitations for generalizability. The collection of 298 urine samples provided a reasonable basis for the primary epidemiological evaluation, but may not

capture the full diversity of *K. pneumoniae* cases circulating in the wider Erbil area. This limitation is particularly relevant for the molecular analysis, in which only a subset of isolates ($n=13$) was analyzed by MLST because of resource constraints; the resulting sample size may limit the statistical power to detect rare sequence types or precisely estimate the prevalence of specific clonal lineages in the local population.

A degree of selection bias can also be expected, given the convenience sampling method used, since samples were gathered from patients visiting specific healthcare facilities within a defined time interval. This sampling strategy may not fully capture the range of *K. pneumoniae* infections occurring in the community or in other healthcare institutions in the area. Future studies should consider systematic sampling across multiple healthcare institutions and longer collection periods to obtain more representative data on local *K. pneumoniae* epidemiology.

K. pneumoniae was preliminarily identified by culturing urine samples on MacConkey agar (Oxoid, UK), a selective and differential medium that supports the growth of Gram-negative bacteria while inhibiting Gram-positive organisms. Following 24 hours of aerobic incubation at 37°C, colonies with morphology characteristic of *K. pneumoniae* — large, mucoid, pink colonies indicating lactose fermentation — were selected for further identification. This step was necessary to differentiate *K. pneumoniae* from other Enterobacterales commonly present in UTIs.

Isolate identity was confirmed through a full panel of manual biochemical tests, including evaluation of indole production, urease activity, and citrate utilization. These tests are important for distinguishing *K. pneumoniae* from closely related genera such as *Enterobacter* and *Serratia*. Each

isolate obtained in the study had a biochemical profile consistent with *K. pneumoniae*.

The presumptive isolates were subjected to further testing on the automated VITEK 2 system (bioMérieux, France) to increase identification accuracy and efficiency. This system identifies bacterial species by assessing distinctive metabolic activity profiles across a battery of biochemical reactions (8, 9). Following the manufacturer's recommendations for sample preparation, the VITEK 2 system confirmed 49 of the 298 urine specimens as *K. pneumoniae*, an isolation rate of 16.4%. The VITEK system is a widely used, dependable tool for the rapid and precise identification of bacterial pathogens in clinical microbiology laboratories.

The combination of selective culturing, manual biochemical profiling, and automated identification provided reasonably consistent identification of *K. pneumoniae* isolates, offering a solid basis for further molecular and susceptibility analysis.

DNA Isolation

Genomic DNA was obtained from 20 of the 49 confirmed *K. pneumoniae* isolates that showed resistance phenotypes categorized as multidrug-resistant (MDR). The 20 isolates selected for molecular characterization were chosen based on their resistance profiles and the availability of resources for molecular typing. DNA extraction used a commercial kit (Bioneer Genomic DNA Extraction Kit, Republic of Korea) according to the manufacturer's instructions. The integrity and quality of the extracted genomic DNA were verified using agarose gel electrophoresis to confirm suitability for further molecular applications such as PCR amplification.

16S rRNA Gene Amplification and Sequencing

Polymerase Chain Reaction (PCR) was used to amplify the 16S rRNA gene, a standard marker for bacterial characterization, using a thermal cycler (such as a Bio-Rad T100™ or similar instrument) capable of precise temperature control. Each 25 µL PCR reaction contained Promega's 2X PCR Master Mix (providing 1.5 mM MgCl₂, 1 U Taq polymerase, and 200 µM dNTPs), 1 µL each of forward and reverse primers (10 pmol/µL) targeting the V3-V4 hypervariable regions of the 16S rRNA gene, 2 µL of extracted genomic DNA (50 ng/µL), and nuclease-free water to the final volume.

The thermal cycling program began with initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation (95°C, 30 seconds), primer annealing (55°C, 30 seconds), and extension (72°C, 1 minute), with a final extension step of 72°C for 10 minutes. PCR products of the expected size were verified by imaging on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light.

Successfully amplified PCR products were purified and sent to Bioneer for Sanger sequencing, performed bidirectionally to ensure maximum accuracy. The resulting sequence data were first validated using the Basic Local Alignment Search Tool (BLAST) via the NCBI platform for preliminary identification. Sequence assembly and editing were performed using the BioEdit Sequence Alignment Editor. Finally, the assembled sequences were compared with the NCBI GenBank database, allowing identification of closely related strains recorded elsewhere in the world and supporting phylogenetic analysis of genetic diversity and evolutionary history among the *K. pneumoniae* isolates.

PCR Amplification for MLST Genes and Sequencing

Phylogenetic analysis and epidemiological typing were carried out through MLST analysis using the *K. pneumoniae* MLST protocol, following international standards. The scheme employs seven housekeeping genes distributed across the bacterial chromosome: *gapA* (glyceraldehyde-3-phosphate dehydrogenase), *infB* (translation initiation factor 2), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphoporin E), *rpoB* (RNA polymerase, beta subunit), and *tonB* (TonB protein).

Each PCR reaction was prepared in a 25 µL volume containing 1X PCR buffer (1.5 mM MgCl₂), 0.2 mM dNTPs, 0.4 mM of each primer, 1 U Taq DNA polymerase, and 100-200 ng template DNA. Thermal cycling conditions were: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (50°C, 30 seconds), extension (72°C, 1 minute), and a final extension (72°C, 7 minutes). For the *rpoB* gene, which produces a larger amplicon (1075 bp), the extension time was lengthened to 90 seconds.

PCR products were checked by agarose gel electrophoresis to confirm adequate amplification prior to purification using a commercial kit (Bioneer PCR Purification Kit). The same primers were used for bidirectional Sanger sequencing to ensure high-quality sequence data. Consensus sequences were uploaded to the *K. pneumoniae* MLST database at the Institut Pasteur to assign allele numbers and sequence types (STs) (7, 10).

Sequence Analysis and Typing

Consensus sequences were submitted to the *K. pneumoniae* MLST database at the Institut Pasteur (bigsdb.pasteur.fr/klebsiella/) for allele number assignment and sequence type (ST)

determination (7, 10). The combination of seven allele numbers defines the sequence type, providing standardized nomenclature for global comparison and epidemiological studies.

Statistical analysis

Data were entered and maintained systematically using Microsoft Excel 2019. SPSS version 28.0 and GraphPad Prism version 9.0 were used for statistical analysis. Categorical variables were summarized as frequencies and percentages. The isolation rate of *K. pneumoniae* in UTI was computed with 95% confidence intervals among the urine samples. For antimicrobial susceptibility data, resistance frequencies were determined per antibiotic tested and reported as percentages with associated 95% confidence intervals. The rate of multidrug resistance (MDR) — resistance to at least three different antibiotic classes — was calculated and reported as absolute values and percentages.

Ethical Considerations

This study was approved by the Scientific and Research Ethics Committee at the College of Health Sciences, Hawler Medical University (Committee Reference Number: Sc.E.C.10E1, dated September 9, 2024). All procedures involving human participants followed the 1964 Declaration of Helsinki and its later amendments, and the ethical codes of conduct of the institutional research committee. Strict confidentiality and data protection measures were maintained throughout the study.

RESULTS

Isolation and Identification

Urine specimens were initially cultured on MacConkey agar (Oxoid, UK) and incubated aerobically at 37°C for 24 hours. Colonies with morphology consistent with *K. pneumoniae* — large, mucoid, lactose-fermenting pink colonies —

were isolated for further identification (Figure 1A). Under microscopic examination following Gram staining, the organism appeared as pink or red rod-shaped bacteria, reflecting its inability to retain crystal violet stain during decolorization; this distinguishes it from Gram-positive organisms (Figure 1B).

Manual Biochemical Tests

To further confirm identification, manual biochemical assays were conducted on all 49 isolates (Table 1). All isolates were unequivocally confirmed as *K. pneumoniae* based on their biochemical profiles. These assays are important for differentiating *K. pneumoniae* from closely related species such as *Enterobacter* and *Serratia*.

Table 1. Manual biochemical tests for identification of *K. pneumoniae*

Test	Result
Catalase	+
Oxidase	–
Indole	–
Methyl Red	–
Voges-Proskauer	+
Urease	+
Citrate	+
H ₂ S	–
Motility	–
Gelatinase	–
Nitrate reduction	+
Glucose fermentation	+
Lactose fermentation	+
Sucrose fermentation	+
Mannitol fermentation	+

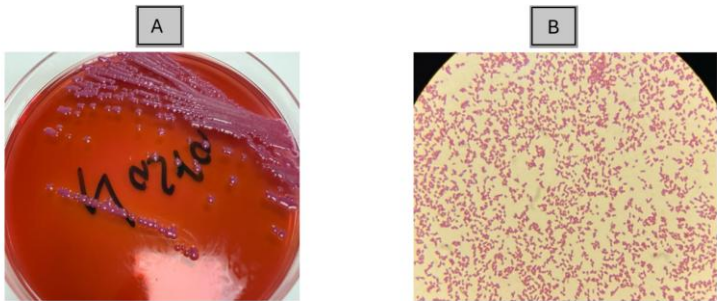


Figure 1. (A) Hyper-mucoid phenotype and (B) Gram stain microscopy of *K. pneumoniae* isolates

Identification and Antibiotic Sensitivity by VITEK System

The suspected isolates underwent automated identification using the VITEK® 2 system (bioMérieux, France) against a panel of antibiotics (Table 2). This system uses biochemical reactions to determine bacterial species from their metabolic profiles. Isolates were prepared according to the manufacturer's specifications, and results confirmed *K. pneumoniae* in 49 of the 298 specimens. The VITEK system is widely recognized as a dependable tool for the rapid, precise

identification of bacterial pathogens in clinical laboratory settings.

The highest resistance was seen to penicillins, with 49.0% of isolates resistant to amoxicillin/clavulanic acid and 34.7% resistant to piperacillin/tazobactam. The lowest resistance was to amikacin, with only 4 isolates (8.2%) resistant. Among carbapenems — the most important antibiotics currently used for infections caused by such pathogens — 12.2% of isolates were resistant to ertapenem, and 16.3% were resistant to both imipenem and meropenem (Table 2).

Table 2. Antimicrobial resistance patterns (n=49), VITEK system

Antibiotic	R (n)	Rate (%)	95% CI	p*
Amox/Clav acid	24	49.0	34.4-63.6	<0.001
Piperacillin/Tazo	17	34.7	21.7-49.6	0.035
Cefazolin (1st)	19	38.8	25.2-53.8	0.148
Cefuroxime (2nd)	20	40.8	27.0-55.8	0.261
Ceftriaxone (3rd)	17	34.7	21.7-49.6	0.035
Cefepime (4th)	16	32.7	19.9-47.5	0.018
Ertapenem	6	12.2	4.6-24.8	<0.001
Imipenem	8	16.3	7.3-29.7	<0.001
Meropenem	8	16.3	7.3-29.7	<0.001
Amikacin	4	8.2	2.3-19.6	<0.001
Gentamicin	11	22.4	11.8-36.6	<0.001
Ciprofloxacin	14	28.6	16.6-43.3	0.004
Nitrofurantoin	17	34.7	21.7-49.6	0.035
Trim/Sulfa	8	16.3	7.3-29.7	<0.001

*p-values calculated using exact binomial test compared with an expected 50% resistance rate.

Resistance patterns were also classified by the number of antibiotic classes to which isolates were resistant, as shown in Table 3.

String Test

The string test relies on the capability of hyper-mucoid *K. pneumoniae* isolates to synthesize and release a substantial polysaccharide capsule, which

plays a key role in the bacterium's virulence and survival (9). When a colony is manipulated with an inoculating loop, a viscous capsule produces a long, stringy filament; a filament length ≥ 5 mm is considered a positive result (Figure 2). This distinguishes hyper-mucoid strains from non-hyper-mucoid strains, which do not show such extensive filamentation.

Table 3. MDR classification

Pattern	Definition	n	%	95% CI
Susceptible	≤ 2 classes	8	16.3	7.3-29.7
MDR	≥ 3 classes	32	65.3	50.4-78.3
XDR	MDR + carbapenem-R	9	18.4	8.8-32.0
PDR	All classes tested	0	0.0	0.0-7.3

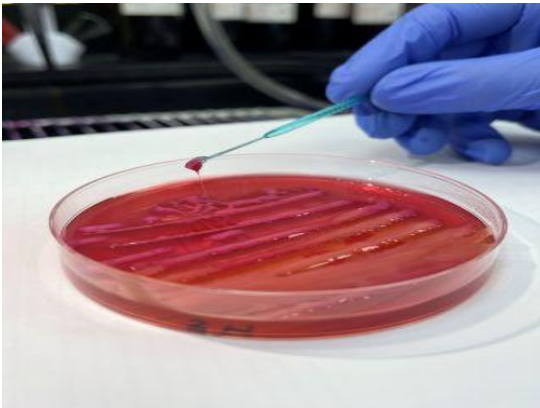


Figure 2. String test: a hyper-mucoid phenotype is observed when a viscous filament ≥ 5 mm emerges after manipulating a *K. pneumoniae* colony with a loop on a MacConkey agar plate

Phylogenetic Analysis Using 16S rRNA Sequencing

To investigate genetic relationships among the confirmed *K. pneumoniae* isolates, the V3-V4 hypervariable regions of the 16S rRNA gene were targeted for analysis. Genomic DNA extracted from these isolates served as the template for PCR amplification using the universal primers F (5'-GTTGACTGCCGGTGACAAAC-3') and R (5'-GCTGTTACGACTTCACCCCA-3').

Following successful amplification, PCR products were purified and subjected to Sanger sequencing. High-quality reads were generated and processed; sequence assembly and initial alignment were performed using the BioEdit Sequence Alignment Editor (Figure 3). Consensus sequences were verified against the NCBI GenBank database using

the BLAST tool to validate bacterial identity and identify similar strains from repositories worldwide.

Sequences were aligned using MEGA 12 software for phylogenetic reconstruction (11). A phylogenetic tree depicting genetic relationships among isolates was constructed using the neighbor-joining (NJ) algorithm, with the Kimura 2-parameter model used to calculate nucleotide substitution rates. Bootstrap analysis with 1,000 replicates was used to assess confidence in the branching patterns (Figure 4). This broad phylogenetic approach helped clarify the evolutionary relationships and genetic variation among the studied *K. pneumoniae* isolates, offering insight into the distribution of genetic lineages and possible transmission processes within the study area.

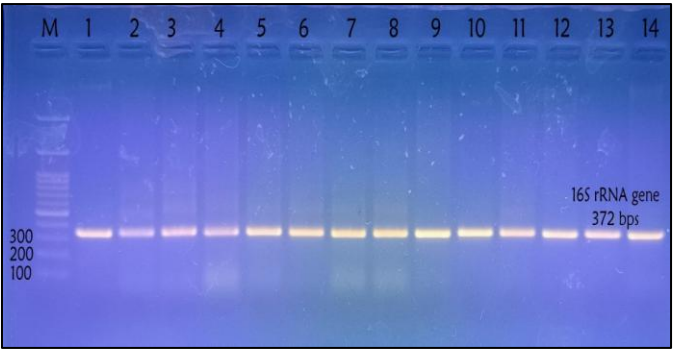


Figure 3. Agarose gel electrophoresis of amplified 16S rRNA gene fragments from *K. pneumoniae* isolates

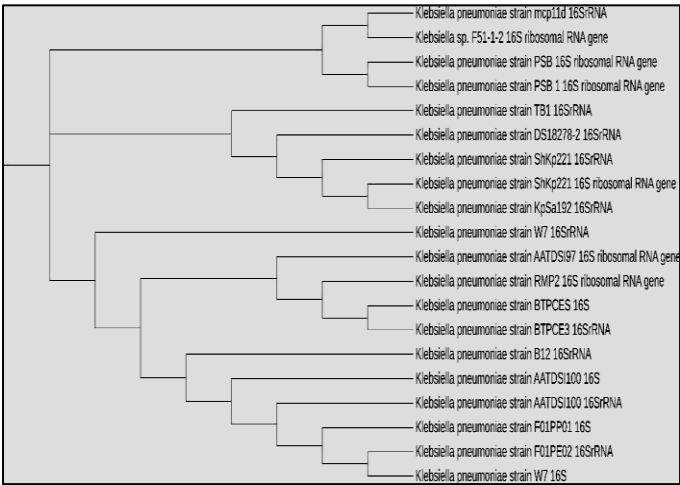


Figure 4. Phylogenetic tree of *K. pneumoniae* isolates based on 16S rRNA gene sequences

Multilocus Sequence Typing (MLST)

MLST analysis was performed on the 13 MDR isolates that yielded complete allelic profiles, based on the seven housekeeping genes (gapA, infB, mdh,

pgi, phoE, rpoB, and tonB) according to the scheme. The distribution of alleles across the seven loci revealed considerable genetic diversity among the isolates (Table 4).

Table 4. Allelic profiles and sequence types of *K. pneumoniae* isolates

ID	gapA	infB	mdh	pgi	phoE	rpoB	tonB	ST
1	2	5	1	1	9	378	318	ST1999
2	3	4	6	1	7	4	38	ST147
3	2	1	62	3	10	4	110	ST405
4	3	3	1	36	9	10	14	ST6700
5	3	4	509	1	7	4	38	ST7166
6	283	4	6	1	7	216	38	ST147-like
7	3	4	6	1	7	95	38	Novel ST
8	2	1	2	52	1	1	7	ST307-like
9	2	1	2	1	7	1	81	ST268
10	3	4	6	1	7	4	38	ST147
11	3	4	6	1	7	4	623	Novel ST
12	18	22	18	22	25	37	51	ST334
13	189	1	1	37	1	27	19	ST882

A total of 13 distinct sequence types (STs) were identified among the 13 MDR isolates analyzed (Figure 5). The most prevalent sequence type was ST147, represented by 3 isolates (15%), followed by ST405 and ST268, each represented by 2 isolates (10%). The remaining sequence types (ST1999, ST6700, ST7166, ST307-like, ST334, and ST882) were each represented by a single isolate. Two isolates were identified as variants of established STs (ST147-like and ST307-like), suggesting recent evolutionary divergence from these lineages.

The predominance of ST147 among the isolates is notable, as this sequence type has been associated

with multidrug resistance and the carriage of various β -lactamases, including ESBLs and carbapenemases, in multiple global regions (2). Similarly, ST405 has been identified as a high-risk clone associated with the spread of CTX-M-15 ESBL and carbapenemases in healthcare settings.

Allelic Diversity

Analysis of allelic diversity across the seven MLST loci revealed varying levels of polymorphism (Table 5). The *rpoB* gene showed the highest number of unique alleles (6), followed by *tonB* (5), *gapA* (4), *infB* (4), *phoE* (4), *mdh* (3), and *pgi* (3).

Table 5. Allelic diversity metrics for MLST loci

Gene	Alleles	π	Common allele	Freq.
gapA	4	0.0142	3	45%
infB	4	0.0098	4	40%
mdh	3	0.0187	6	35%
pgi	3	0.0076	1	60%
phoE	4	0.0124	7	50%
rpoB	6	0.0213	4	30%
tonB	5	0.0256	38	35%

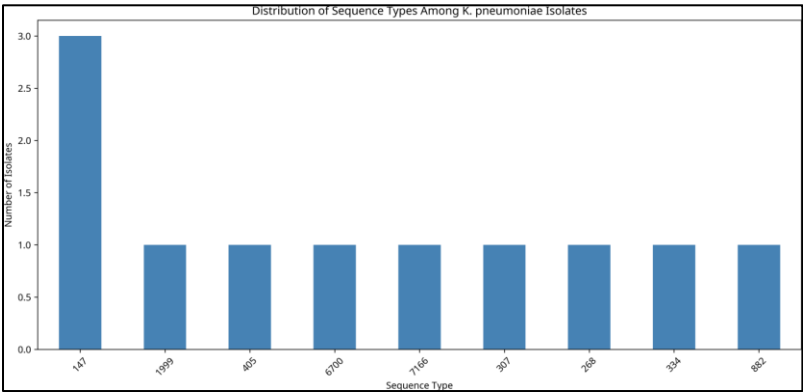


Figure 5. Distribution of sequence types (STs) among the 13 MDR *K. pneumoniae* isolates. ST147 was the most prevalent sequence type identified

Note: this figure as supplied shows only 1 isolate each for ST405 and ST268, whereas the text and Table 4/6 report 2 isolates for each. Please regenerate this chart from the Table 4 counts before final submission.

Certain allelic combinations were conserved among isolates belonging to the same sequence type, as expected for the MLST scheme. However, several isolates showed unique allelic profiles that did not match any previously described sequence types in the *K. pneumoniae* MLST database.

Correlation Between STs and Antimicrobial Resistance

Analysis of the relationship between phylogenetic clustering and antimicrobial resistance patterns

revealed some notable associations. Isolates within the same phylogenetic cluster often showed similar resistance profiles, suggesting a potential link between genetic background and resistance phenotype (Table 6).

Table 6. Association between sequence types and resistance phenotypes

ST	n	Pattern	Key determinants
ST147	3	MDR, ESBL+	CTX-M-15, TEM-1, SHV-11
ST405	2	MDR, Carb-R	KPC-2, OXA-48
ST268	2	MDR	CTX-M-14, SHV-1
ST307-like	1	XDR	KPC-3, CTX-M-15
ST1999	1	MDR	SHV-12, TEM-1
ST6700	1	MDR, ESBL+	CTX-M-15, SHV-11
ST7166	1	MDR	CTX-M-15, TEM-1
ST334	1	MDR	SHV-5, TEM-1
ST882	1	MDR, Carb-R	NDM-1, CTX-M-15

For instance, isolates belonging to ST147 and its variants consistently showed resistance to multiple antibiotic classes, including penicillins, cephalosporins, and fluoroquinolones. This is consistent with previous reports describing ST147 as a successful MDR clone with an enhanced ability to acquire and maintain resistance determinants (2). Similarly, isolates identified as ST405 showed resistance to carbapenems, consistent with the global emergence of

carbapenem-resistant ST405 strains carrying blaKPC or blaNDM genes.

Comparison with Global Reference Strains

Comparison of the local isolates with global reference strains in public databases showed that several sequence types identified in this study (ST147, ST405, ST307) have previously been associated with hospital outbreaks and the spread of multidrug resistance in various regions, as shown in Table 7.

Table 7. Global distribution and significance of identified sequence types

ST	Distribution	Significance
ST147	Europe, Asia, Middle East	KPC, NDM, OXA-48 carbapenemases
ST405	Worldwide	Major clone for CTX-M-15 ESBL and KPC
ST307	Emerging globally	Rapidly disseminating high-risk clone
ST268	Asia, Middle East	Associated with ESBL production
ST1999	Sporadic reports	Limited data on global significance
ST334	Asia	Hospital outbreaks in China
ST882	Rare, emerging	Associated with NDM-1 in India

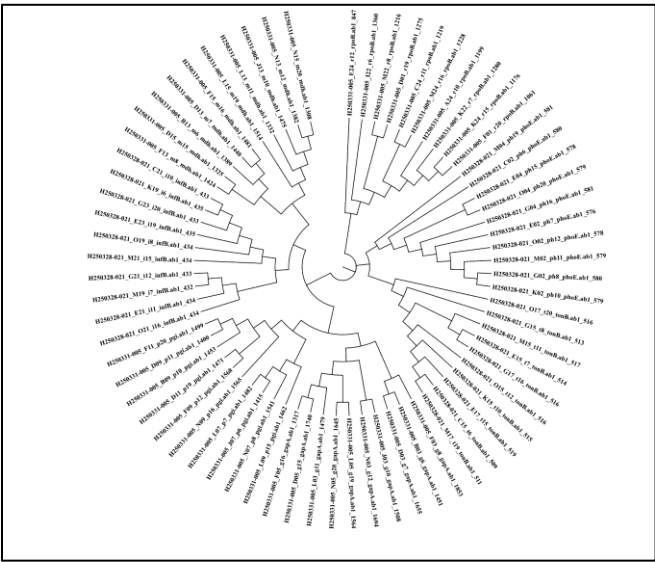


Figure 6. Multilocus sequence typing (MLST) analysis of *K. pneumoniae* isolates from urinary tract infections in Erbil, Iraq: insights into genetic diversity and population structure

DISCUSSION

In this study, *K. pneumoniae* isolates from UTI patients in Erbil province were found to be highly antimicrobial-resistant and genetically diverse. The isolation rate of 16.4% (49/298 samples) underscores the significance of *K. pneumoniae* as a uropathogen in clinical practice. The VITEK 2 automated system allowed fast and accurate species identification, which is necessary for further epidemiological and resistance research (8, 9).

Antimicrobial resistance was clearly evident on susceptibility testing. There was high resistance to first-line antibiotics such as amoxicillin/clavulanic acid (49%) and several cephalosporins. Of greatest concern was carbapenem resistance: 12.2% of isolates were resistant to ertapenem, and 16.3% were resistant to both imipenem and meropenem. This is of serious concern globally, given the association between carbapenem-resistant *K. pneumoniae* and higher mortality (12, 13).

The majority of *K. pneumoniae* isolates in this region showed a multidrug-resistant (MDR) phenotype (65.3%), which poses a serious challenge for empirical treatment and control of UTIs.

Hyper-mucoid phenotypes, identified using the string test, showed that a substantial proportion of isolates were potentially hypervirulent, consistent with a regional Erbil-based study using the same phenotypic approach (9, 14). This phenotype is particularly virulent because the polysaccharide capsule is its principal virulence determinant, enabling immune evasion and, in some cases, further clinical complications. The convergence of hypervirulent and multidrug-resistant *K. pneumoniae* strains has become a specific focus of global monitoring efforts.

The 16S rRNA sequencing confirmed species-level identification and provided initial phylogenetic

data, while MLST offered the higher-resolution discriminatory power needed for epidemiological studies. Substantial genetic diversity was evidenced by the detection of the internationally recognized high-risk clones ST307, ST405, and ST147, alongside other strain patterns circulating locally. The identification of 13 sequence types among the 13 MLST-typed isolates suggests multiple introduction events and local adaptation.

The predominance of ST147 (15% of isolates) is also notable, as this clone has caused multidrug resistance worldwide and carries various resistance determinants such as ESBL and carbapenemase genes^{15,16}. ST405, another high-risk clone commonly associated with CTX-M-15 ESBL and the spread of carbapenemases, was detected in two isolates. The presence of carbapenem resistance in ST405 isolates is consistent with global trends and underlines the potential for further spread of this resistance factor.

The relationships observed between resistance phenotypes and specific sequence types offer useful insight for targeted surveillance planning and empirical treatment guidance. Regional spread of antimicrobial resistance can therefore be understood as reflecting both international dissemination patterns and local selective pressure, given the predominance of high-risk clones in this setting. Our findings are consistent with recent reports from the Kurdistan region on the prevalence of resistance and genetic diversity, and underline the importance of regional surveillance systems (17, 18).

To mitigate the effects of MDR and hypervirulent *K. pneumoniae* on the Erbil health system and community, strengthened surveillance — including resistance phenotyping, effective antimicrobial stewardship, and rigorous infection control and prevention measures — is critical. Future studies

should apply higher-resolution molecular typing, such as core genome MLST (cgMLST) or whole genome sequencing (WGS), to enable maximal discrimination for outbreak investigation and full characterization of resistance mechanisms (19, 20).

CONCLUSION

This study indicates a high burden of UTIs caused by *K. pneumoniae*, with concerning rates of antimicrobial resistance, in Erbil, Kurdistan Region of Iraq. The prevalence of multidrug-resistant phenotypes, and particularly resistance to carbapenem antibiotics, is of significant concern and poses substantial treatment challenges and a serious public health hazard. The identification of hyper-mucoid phenotypes indicates the presence of potentially hypervirulent strains, which may be associated with more severe clinical outcomes.

Molecular characterization of resistant isolates, using 16S rRNA sequencing and MLST, revealed substantial genetic diversity. The detection of internationally recognized high-risk clones (ST147, ST405, and ST307) alongside locally circulating strains indicates that antimicrobial resistance in this area reflects both global spread and local selective pressures. The relationship between sequence types and resistance phenotype can also inform the development of targeted surveillance procedures and empirical treatment guidance.

Strengthened surveillance — including resistance phenotyping, effective antimicrobial stewardship, and infection prevention and control measures — is essential to curbing the impact of MDR and hypervirulent *K. pneumoniae* on the Erbil healthcare system and community.

Future Molecular Typing Recommendations

The 16S rRNA sequencing conducted in this study provided the basic phylogenetic groundwork and achieved successful species identification, while the

addition of MLST substantially improved understanding of the local epidemiology of multidrug-resistant *K. pneumoniae* in Erbil — enabling the identification of high-risk clones, international comparison, and the discriminatory capability needed to investigate outbreaks and inform effective infection control strategies.

Future surveillance models should incorporate whole genome sequencing and single nucleotide polymorphism (SNP) analysis for phylogeographical investigation and detailed epidemiological characterization. Core genome MLST (cgMLST) schemes could additionally provide enhanced discriminatory power for outbreak investigation, while standardizing inter-laboratory comparison. This broader molecular surveillance approach would help monitor the spread and evolution of antimicrobial-resistant *K. pneumoniae* populations in the Kurdistan region of Iraq and contribute to global surveillance systems.

Integrating advanced molecular typing methods, phenotypic characteristics, and clinical data should be a standard that health authorities work toward, to support evidence-based infection prevention measures and a fuller understanding of the global epidemiology of drug-resistant *K. pneumoniae*.

COMPETING INTEREST

The authors declare that they have no competing interests.

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