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Research Article

Multilocus Sequence Typing Reveals Diverse Clonal Lineages of *Klebsiella pneumoniae* Causing Urinary Tract Infections

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RDJA: Conceptualization, methodology, laboratory investigation, data collection, molecular analysis, data interpretation, and manuscript drafting. MKAA: Supervision, study design, data analysis, manuscript revision, and final approval of the manuscript.

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Klebsiella pneumoniae, UTI, MLST, Sequence types, MDR, Metallo-β-lactamase genes

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Abstract | *Klebsiella pneumoniae* is a major uropathogen, particularly in healthcare settings, where multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains pose significant therapeutic challenges. Understanding the population structure and genetic diversity of *K. pneumoniae* is essential for effective infection control and clinical management. In this study, multilocus sequence typing (MLST) was performed to characterize 35 *K. pneumoniae* isolates recovered from patients with urinary tract infections (UTIs). Seven conserved housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) were amplified and sequenced to determine sequence types (STs) and clonal complexes (CCs). The isolates were classified into five sequence types: ST15, ST307, ST258, ST512, and ST101, which clustered into three major clonal complexes (CC15, CC258, and CC101). Antimicrobial susceptibility testing revealed high rates of multidrug resistance. MDR isolates were mainly associated with ST15 (CC15) and ST307 (CC258), whereas XDR isolates predominantly belonged to ST258 and ST512 (CC258) and ST101 (CC101). Molecular analysis of metallo-β-lactamase genes showed the presence of blaNDM and blaVIM among carbapenem-resistant isolates. Phylogenetic analysis based on MLST loci demonstrated close genetic relatedness within clonal complexes, alongside allelic variability suggesting ongoing genetic diversification. These findings highlight the circulation of high-risk international clones among UTI-associated *K. pneumoniae* isolates and emphasize the importance of continuous molecular surveillance to guide infection control strategies and antimicrobial stewardship programs.

Novelty Statement | This study highlights the genetic diversity of *Klebsiella pneumoniae* isolates associated with urinary tract infections using multilocus sequence typing. High-risk international clones linked to multidrug-resistant and extensively drug-resistant phenotypes were identified among the studied isolates. The findings emphasize the importance of continuous molecular surveillance and antimicrobial stewardship to control the spread of resistant strains.

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Introduction

Klebsiella pneumoniae is a Gram-negative, encapsulated bacterium belonging to the family Enterobacteriaceae (Jin *et al.*, 2025). It is an opportunistic pathogen responsible for a wide range of hospital-acquired and community-acquired infections, including pneumonia, septicemia, and urinary tract infections (UTIs) (Fekadu *et al.*, 2025). *K. pneumoniae* is a significant cause of UTIs, particularly among hospitalized and immunocompromised patients, where infections may lead to severe complications and increased morbidity and mortality (Azoulay *et al.*, 2020). The emergence of multidrug-resistant (MDR) and

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extensively drug-resistant (XDR) strains has become a major public health concern due to increasing challenges in infection control and effective antimicrobial therapy (Wyres and Holt, 2018). These resistant strains harbor multiple resistance mechanisms, including the production of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, and carbapenemases, which confer resistance to several antibiotic classes, including carbapenems (Sawa *et al.*, 2020).

The epidemiology and population structure of *K. pneumoniae* are fundamental for implementing effective infection control policies and guiding antimicrobial therapy. Molecular typing techniques, particularly multilocus sequence typing (MLST), have proven valuable for assessing the population structure and transmission dynamics of *K. pneumoniae* (Elias *et al.*, 2025). MLST is a standardized and reproducible method based on sequencing internal fragments of seven conserved housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) (Diancourt *et al.*, 2005). The combination of alleles at these loci defines a sequence type (ST), which enables global comparison of circulating clones and their evolutionary relationships (Dong *et al.*, 2025). Closely related STs differing at a single locus variant (SLV) are grouped into clonal complexes (CCs), providing insights into genetic relatedness and lineage diversification (Brisse *et al.*, 2009).

Several studies have investigated the antimicrobial resistance and susceptibility patterns of *K. pneumoniae* isolates from diverse clinical and geographic settings (Aslam *et al.*, 2025). These investigations have revealed the widespread dissemination of high-risk international clones, particularly ST258, which is frequently associated with carbapenem resistance and the production of New Delhi metallo-β-lactamase (NDM) (Koli *et al.*, 2025). Other clinically important sequence types, including ST11, ST15, and ST307, have also been linked to the global spread of multidrug resistance (Ito *et al.*, 2025).

K. pneumoniae is a major causative agent of UTIs, and understanding its genotypic diversity and antimicrobial resistance patterns is essential for effective clinical management and infection control (Aiesh *et al.*, 2025). Previous studies have demonstrated the value of MLST in characterizing UTI-associated *K. pneumoniae* isolates and tracking the dissemination of resistant clones (Kimunya, 2025).

Therefore, the present study aimed to analyze the genetic characteristics of 35 *K. pneumoniae* isolates recovered from UTI patients using MLST. Specifically, we sought to: (1) determine their sequence types and clonal complexes; (2) assess their antimicrobial susceptibility profiles; and (3) detect metallo-β-lactamase genes associated with carbapenem resistance. The findings provide insight into the molecular epidemiology and resistance landscape of UTI-associated *K. pneumoniae*, supporting targeted infection control strategies and evidence-based therapeutic decisions.

Materials and Methods

Bacterial isolates and antimicrobial susceptibility testing

A total of 35 non-duplicate *Klebsiella pneumoniae* isolates were recovered from female patients diagnosed with urinary tract infections (UTIs) attending several hospitals in Babil Governorate, Iraq, including Al-Shomali General Hospital, Al-Hashimiya General Hospital, Al-Qasim General Hospital, Maternity and Children Hospital, Marjan Teaching Hospital, and Imam Ali Al-Sadiq Teaching Hospital. Urine samples were collected between December 15, 2024 and June 15, 2025, and processed according to standard microbiological procedures. Bacterial identification was performed based on colony morphology, Gram staining, and conventional biochemical tests.

Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antimicrobial agents were tested: amoxicillin-clavulanate (AMC), ticarcillin-clavulanate (TIM), ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX), ceftaroline-avibactam (CPA), aztreonam (ATM), imipenem (IPM), meropenem (MEM), amikacin (AN), ciprofloxacin (CIP), levofloxacin (LVX), chloramphenicol (C), ceftolozane-tazobactam (CT), tigecycline (TGC), and piperacillin-tazobactam (TZP). Interpretations of susceptibility results were made according to CLSI breakpoints.

Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) was performed according to the protocol described by Diancourt *et al.* (2005). Internal fragments of seven housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) were amplified by polymerase chain reaction (PCR) using the primers listed in Table 1.

PCR amplification was carried out in a final reaction volume of 25 µL containing genomic DNA template, 10 pmol of each primer, PCR master mix, and nuclease-free water. The amplification conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30–60 sec (depending on the target gene), and extension at 72°C for 30 sec, with a final extension at 72°C for 5 min.

PCR products were purified and sequenced in both directions. The obtained sequences were analyzed and compared with the *K. pneumoniae* MLST database to

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Table 1: Primers used for MLST of *Klebsiella pneumoniae*.

Genes	Direction	Primer sequence
rpoB	F: Vic3	GGCGAAATGGCWGAGAACCA
	R: Vic2	GAGTCTTCGAAGTTGTAACC
gapA	F: gapA173	TGAAATATGACTCCACTCACGG
	R: gapA181	CTTCAGAAGCGGCTTTGATGGCTT
Mdh	F: mdh130	CCCAACTCGCTTCAGGTTCAG
	R: mdh867	CCGTTTTTCCCCAGCAGCAG
Pgi	F: pgi1F	GAGAAAAACCTGCCTGTACTGCTGGC
	R: pgi1R	CGCGCCACGCTTTATAGCGGTTAAT
phoE	F: phoE604.1	ACCTACCGCAACACCGACTTCTTCGG
	R: phoE604.2	TGATCAGAACTGGTAGGTGAT
infB	F: infB1F	CTCGCTGCTGGACTATATTCG
	R: infB1R	CGCTTTCAGCTCAAGAACTTC
tonB	F: tonB1F	CTTTATACCTCGGTACATCAGGTT
	R: tonB2R	ATTCGCCGGCTGRGCRGAGAG

assign allele numbers and determine sequence types (STs). Clonal complexes (CCs) were defined based on single-locus variants (SLVs).

Data analysis

Allele sequences obtained from MLST were compared with the *Klebsiella pneumoniae* MLST database to assign allele numbers and determine sequence types (STs). Clonal complexes (CCs) were defined based on single-locus variants (SLVs). Multiple sequence alignments were performed using the ClustalW algorithm implemented in MEGA version 11.0.13. Phylogenetic relationships were inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on concatenated sequences of the seven housekeeping genes. The resulting phylogenetic tree was visualized and edited using MEGA version 11.0.13.

Results

Klebsiella pneumoniae *susceptibility analysis*

Antimicrobial susceptibility testing of the 35 *Klebsiella pneumoniae* isolates revealed high resistance rates to β-lactam antibiotics, including amoxicillin–clavulanate, ticarcillin–clavulanate, cefotaxime, ceftazidime, and piperacillin–tazobactam (Table 2). All isolates were resistant to fosfomycin.

Carbapenem resistance was observed in a substantial proportion of isolates, particularly for imipenem, whereas meropenem retained comparatively higher activity. Aminoglycosides, especially amikacin, demonstrated strong effectiveness against most isolates. Resistance to fluoroquinolones varied markedly, with high resistance to ciprofloxacin but lower resistance to levofloxacin. Tigecycline and ceftolozane–tazobactam also exhibited favorable activity profiles.

Antibiotic resistance pattern

Based on established international definitions, the isolates were categorized into multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) phenotypes (Table 3). Among the 35 *Klebsiella pneumoniae* isolates, 6 (17.14%) were classified as MDR, while 29 (82.86%) were categorized as XDR. No isolates met the criteria for PDR. MDR isolates exhibited resistance to at least three antimicrobial classes but retained susceptibility to selected agents such as meropenem, amikacin, or levofloxacin. In contrast, XDR isolates demonstrated resistance to nearly all tested antibiotic classes, remaining susceptible only to a limited number of therapeutic options.

Table 2: Antimicrobial susceptibility profiles of *Klebsiella pneumoniae* Isolates (n= 35).

Antimicrobial Agent	Resistant (R)	Intermediate (I)	Susceptible (S)
Amoxicillin-clavulanate (AMC)	35(100%)	0 (0%)	0 (0%)
Ticarcillin-clavulanate (TIM)	35(100%)	0 (0%)	0 (0%)
Ceftriaxone (CRO)	34 (97%)	1 (3%)	0 (0%)
Ceftazidime (CAZ)	35(100%)	0 (0%)	0 (0%)
Cefotaxime (CTX)	35(100%)	0 (0%)	0 (0%)
Ceftaroline-avibactam (CPA)	35(100%)	0 (0%)	0 (0%)
Aztreonam (ATM)	3 (9%)	0 (0%)	32 (91%)
Imipenem (IPM)	29 (83%)	0 (0%)	6 (17%)
Meropenem (MEM)	5 (14%)	1 (3%)	29 (83%)
Amikacin (AN)	1 (3%)	0 (0%)	34 (97%)
Ciprofloxacin (CIP)	33 (94%)	0 (0%)	2 (6%)
Levofloxacin (LVX)	2 (6%)	0 (0%)	33 (94%)
Chloramphenicol (C)	0 (0%)	0 (0%)	35(100%)
Ceftolozane-tazobactam (CT)	0 (0%)	3 (9%)	32 (91%)
Tigecycline (TGC)	2 (6%)	0 (0%)	33 (94%)
Fosfomycin (FOS)	35(100%)	0 (0%)	0 (0%)
Piperacillin-tazobactam (TZP)	35(100%)	0 (0%)	0 (0%)

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Table 3: Classification of *Klebsiella pneumoniae* isolates by resistance level.

Resistance pattern	Definition	Number of isolates (n=35)	Key antibiotics resistant
Non-MDR	Susceptible to all or resistant to only 1–2 antibiotic classes	0 (0%)	None (All isolates showed resistance to multiple classes)
MDR (Multidrug Resistant)	Resistant to ≥3 antibiotic classes (e.g., β-lactams, fluoroquinolones, aminoglycosides)	6 (17.14%)	Resistant to: β-lactams (e.g., AMC, CAZ) + CIP but susceptible to MEM, AN, LVX, ATM
XDR (Extensively Drug Resistant)	Resistant to all but 1–2 antibiotic classes (e.g., only susceptible to colistin or tigecycline)	29 (82.86%)	Resistant to: β-lactams (including carbapenems), CIP, FOS; susceptible only to AN, LVX, ATM, CT, TGC
PDR (Pandrug Resistant)	Resistant to all tested antibiotic classes (no susceptibility left)	0 (0%)	None (All isolates showed resistance to multiple classes)

Table 4: The allelic profiles of seven conserved housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB for *Klebsiella pneumoniae*).

Isolate ID	ST	gapA	infB	mdh	pgi	phoE	rpoB	tonB	Clonal complex (CC)
KP-MDR-01	15	1	1	1	1	1	1	1	CC15
KP-MDR -02	307	4	1	2	52	1	1	7	CC258
KP-XDR-03	258	3	1	1	1	1	1	1	CC258
KP-XDR-04	512	54	3	1	1	1	1	79	CC258
KP-XDR-05	101	2	6	1	5	4	1	6	CC101

Table 5: MLST analysis association with resistance profile of *Klebsiella pneumoniae* isolates.

Isolate ID	Sequence type (ST)	Clonal complex (CC)	Resistance profile
KP-MDR-01	ST15	CC15	MDR (Resistant to AMC, CAZ, CIP; Susceptible to MEM, AN, LVX)
KP-MDR-02	ST307	CC258	MDR (Resistant to AMC, CAZ, CIP; Susceptible to MEM, CT, ATM)
KP-XDR-03	ST258	CC258	XDR (Resistant to AMC, CAZ, IPM, CIP; Susceptible only to AN, TGC)
KP-XDR-04	ST512	CC258	XDR (Resistant to AMC, CAZ, MEM, CIP; Susceptible only to LVX, CT)
KP-XDR-05	ST101	CC101	XDR (Resistant to AMC, CAZ, IPM, FOS; Susceptible only to ATM, TGC)

MLST results

MLST analysis identified five distinct sequence types (ST15, ST307, ST258, ST512, and ST101) among the *Klebsiella pneumoniae* isolates (Table 4). These sequence types were grouped into three clonal complexes: CC15, CC258, and CC101. ST15 was assigned to CC15, while ST307, ST258, and ST512 belonged to CC258. ST101 was classified within CC101. The allelic profiles of the seven housekeeping genes are presented in Table 4, and their association with antimicrobial resistance patterns is summarized in Table 5. Phylogenetic analysis based on concatenated MLST loci demonstrated close genetic relatedness among isolates belonging to the same clonal complexes (Figure 1).

Discussion

Rising trend of the multidrug resistant (MDR)/ extended drug resistance (XDR) *Klebsiella pneumoniae* strains has led to a major concern in public health, especially in urinary tract infections (UTI) (Bayaba *et al.*, 2025). The epidemiology and population structure of these resistant *K. pneumoniae* isolates are important to consider to devise successful strategies for infection control and appropriate antimicrobial treatment (Sahoo *et al.*, 2025). In the present study, 35 UTI-associated *Klebsiella pneumoniae* isolates were analyzed to determine their antimicrobial resistance profiles and genetic characteristics using multilocus sequence typing (MLST). MLST identified five distinct sequence types distributed among three major clonal complexes, highlighting the circulation of internationally recognized high-risk lineages within the study setting.

The distribution of MDR and XDR phenotypes among the identified sequence types reflects the well-established association between specific clonal lineages and antimicrobial resistance patterns. In the present study, ST15 and ST307 were predominantly associated with MDR phenotypes, whereas ST258, ST512, and ST101 were mainly linked to XDR profiles.

CC258-related lineages, particularly ST258 and ST512, are recognized as globally disseminated high-risk clones frequently associated with carbapenem resistance and hospital outbreaks (Wyres and Holt, 2018). The detection of these lineages among UTI-associated isolates in the current study highlights their continued clinical relevance and potential for regional spread.

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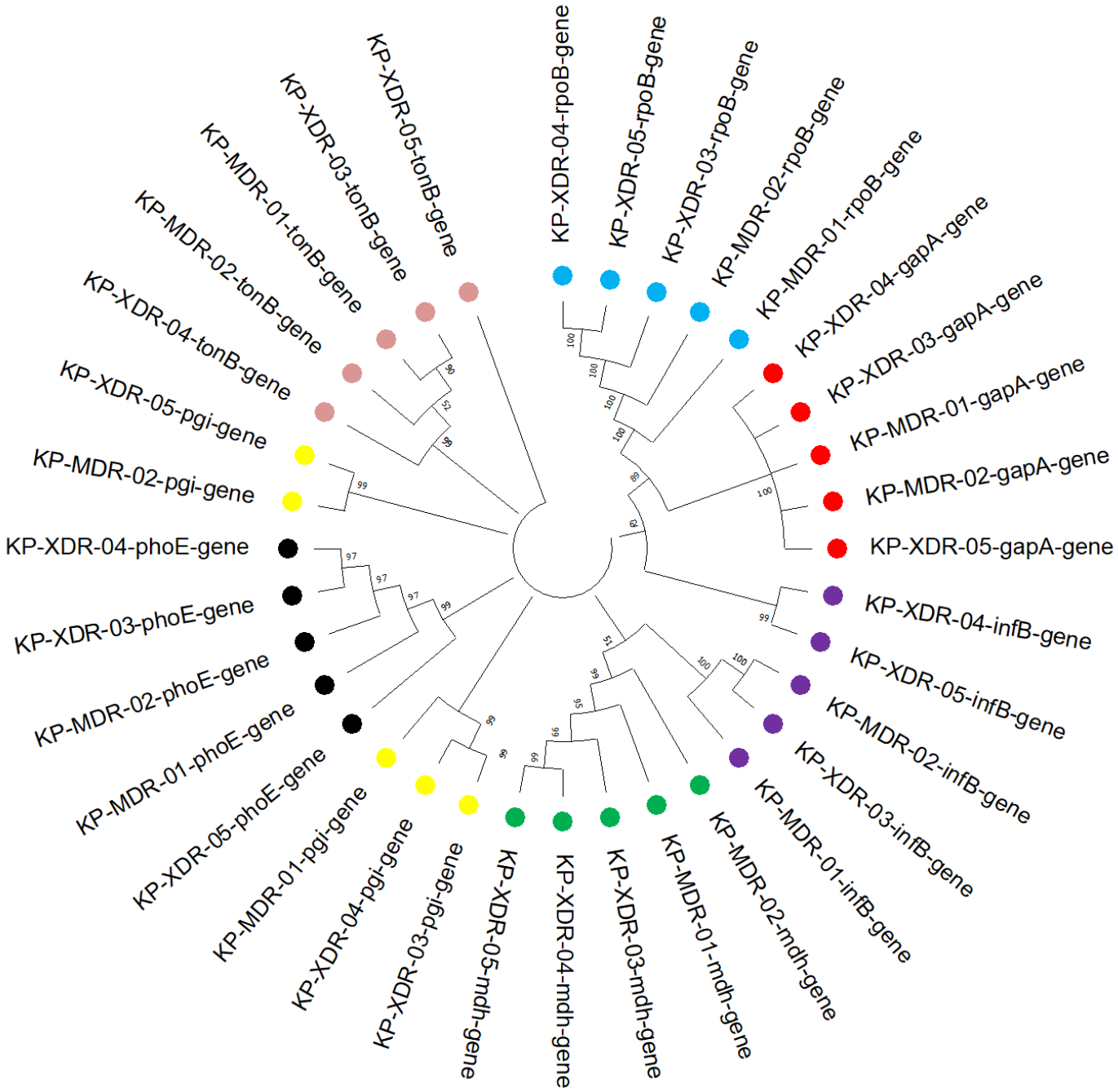

Figure 1: Phylogenetic tree constructed using the UPGMA method based on concatenated sequences of seven MLST housekeeping genes (gapA, infB, mdh, pgi, phoE, rpoB, and tonB). Conserved alleles (infB-1, rpoB-1, mdh-1) were identified across all isolates, whereas polymorphic alleles (gapA, pgi, phoE, and tonB) contributed to genetic differentiation among sequence types.

In the present study, XDR isolates were distributed across multiple sequence types, including ST258 and ST512 (both belonging to CC258) as well as ST101 (CC101). These high-risk lineages demonstrated resistance to nearly all tested antimicrobial classes, remaining susceptible only to limited therapeutic options such as amikacin, tigecycline, and ceftolozane–tazobactam.

The predominance of XDR *Klebsiella pneumoniae* isolates is particularly concerning due to the markedly restricted treatment options available for managing UTI cases caused by these strains (Shah *et al.*, 2025).

The dissemination of ST258 (CC258) and ST101 (CC101) aligns with previous global reports documenting the intercontinental expansion of these high-risk clones associated with carbapenem resistance (Nakamura-Silva *et al.*, 2021).

ST258 is widely recognized as a high-risk international clone strongly associated with carbapenemase production, particularly New Delhi metallo-β-lactamase (NDM) (Mitra *et al.*, 2025; Talat *et al.*, 2024). The detection of ST258 among XDR isolates in the present study is therefore of significant clinical concern, as this lineage

has been implicated in the global dissemination of carbapenem-resistant *Klebsiella pneumoniae* (Wyres and Holt, 2018).

Molecular analysis revealed the presence of blaNDM and blaVIM genes in all 29 carbapenem-resistant isolates. The widespread detection of these carbapenemase genes likely explains the high resistance rates observed for imipenem and other β-lactam antibiotics. Similar findings have been reported in previous regional and international studies describing the rapid spread of NDM-producing *K. pneumoniae* strains (Khalil *et al.*, 2015).

In addition, the identification of ST101 among XDR isolates aligns with earlier reports highlighting this clone as an emerging multidrug-resistant lineage frequently associated with various carbapenemases, including NDM and OXA-type enzymes (Palmieri *et al.*, 2020). The coexistence of high-risk clones and carbapenemase genes underscores the urgent need for enhanced molecular surveillance in clinical settings.

Phylogenetic analysis based on concatenated MLST loci demonstrated clear genetic clustering of isolates within their respective clonal complexes. Conserved alleles such as infB-1, rpoB-1, and mdh-1 were identified across multiple isolates, suggesting a shared evolutionary background. In contrast, variability in alleles of gapA, pgi, phoE, and tonB contributed to differentiation among sequence types, reflecting ongoing genetic diversification within circulating *K. pneumoniae* lineages. These findings support the utility of MLST in distinguishing closely related strains and monitoring clonal dissemination.

The results of this study are consistent with previous studies indicating that K. pneumoniae is among the most common pathogens responsible for UTIs (Bayaba *et al.*, 2025). A study by Rodríguez-Baño *et al.* (2013) similarly reported carbapenem-resistant *K. pneumoniae* isolates, with a high proportion classified as the high-risk ST258 clone, from patients with community-onset UTIs. In a similar study from India, Heidary *et al.* (2018) used MLST to type CR *K. pneumoniae* isolated from UTIs, with most being ST11, another global epidemic and multidrug-resistant clone.

The high prevalence of MDR and XDR *Klebsiella pneumoniae* isolates associated with UTIs in the present study carries significant clinical and public health implications. The limited availability of effective treatment options underscores the urgent need for strengthened infection control measures and optimized antimicrobial stewardship strategies.

Continuous surveillance and molecular epidemiological investigations, including MLST-based analyses, are essential for monitoring the dissemination of high-risk clones and emerging resistance determinants. Targeted infection prevention strategies such as improved hand hygiene, rational antimicrobial use, and active surveillance programs have been shown to reduce the spread of MDR and XDR *K. pneumoniae* in hospital settings (Okeah *et al.*, 2021).

Furthermore, recent cross-disciplinary research suggests that, alongside the development of new antimicrobial agents, optimization of existing therapies and exploration of alternative treatment modalities such as phage therapy or combination regimens may be required to address the growing burden of carbapenem-resistant *K. pneumoniae* infections (Ding *et al.*, 2025). Continued investigation into the epidemiology and population dynamics of UTI-associated *K. pneumoniae* will be crucial for guiding future therapeutic strategies and public health interventions.

Conclusion

This study demonstrates a high prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Klebsiella pneumoniae* among urinary tract infection isolates in the studied region. MLST analysis identified five sequence types clustered into three major clonal complexes, including internationally recognized high-risk lineages such as ST258 and ST101. The consistent detection of carbapenemase genes among resistant isolates highlights the growing therapeutic challenge posed by these strains. These findings emphasize the urgent need for continuous molecular surveillance, strict infection control practices, and optimized antimicrobial stewardship programs to limit the dissemination of high-risk clones and preserve effective therapeutic options.

Declarations

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IRB approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of the University of Al-Qadisiyah, Iraq (Approval No. 298).

Ethical statement

This study was approved by the Institutional Ethics Committee of the University of Al-Qadisiyah (Approval No: 298). The study was conducted in accordance with institutional and national ethical standards. Patient confidentiality was strictly maintained, and no personal identifying information was recorded.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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