

High Prevalence of Urinary Tract Infection-Associated Multidrug- and Extensively Drug-Resistant *Klebsiella pneumoniae* in Riyadh, Saudi Arabia

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Abstract

Multidrug-resistant *Klebsiella pneumoniae*, the pathogen of nosocomial infections, has gained significant clinical importance. The rise of clinical *K. pneumoniae* resistance to ESBL (extended-spectrum beta-lactamase) has complicated the treatment of associated infections. Hospital samples were collected from the Riyadh region and characterized for antimicrobial susceptibility profiles of urinary tract infection-associated *K. pneumoniae*. *K. pneumoniae* isolates (26) were collected from three hospitals. Multiple techniques (API 20E strips, VITEK 2 compact, and 16s rRNA sequencing) were followed for their phenotypic and genotypic confirmation. The VITEK 2 compact system assisted in phenotypic characterization of antimicrobial susceptibility patterns, whereas resistance-encoding genes were detected through PCR amplification. Approximately all *K. pneumoniae* isolates appeared to be ESBL-positive and exhibited MDR patterns. Moreover, a high prevalence of resistance genes (*bla*_{TEM}, *bla*_{KPC}, and *bla*_{OXA-48}) was noted in these isolates. These findings denote a critical resistance threshold necessitating effective antimicrobial stewardship, regular molecular surveillance, and reinforced preventive measures under the integrated One Health framework. This approach could restrict the further spread of environmental, clinical, and community-related multidrug-resistant *K. pneumoniae*.

Keywords: *Klebsiella pneumoniae*, urinary tract infections, antimicrobial resistance, ESBL, resistance genes.

Резюме

Мултирезистентната *Klebsiella pneumoniae*, патогенът на нозокомиалните инфекции, придоби значително клинично значение. Нарастващата клинична резистентност на *K. pneumoniae* към ESBL (разширен спектър бета-лактамаза) усложни лечението на свързаните инфекции. В района на Рияд бяха събрани болнични проби и характеризирани профили на антимикробна чувствителност на *K. pneumoniae*, свързана с инфекция на пикочните пътища. Изолати на *K. pneumoniae* (26) бяха събрани от три болници. За тяхното фенотипно и генотипно потвърждение бяха използвани множество техники (API 20E стрипове, VITEK 2 compact и 16s rRNA секвениране). Компактната система VITEK 2 подпомогна фенотипното характеризиране на моделите на антимикробна чувствителност, докато гените, кодиращи резистентност, бяха открити чрез PCR амплификация. Приблизително всички изолати на *K. pneumoniae* изглеждаха ESBL-позитивни и показваха MDR модели. Освен това, в тези изолати беше наблюдавана висока честота на гени за резистентност (*bla*_{TEM}, *bla*_{KPC} и *bla*_{OXA-48}). Тези открития показват критичен праг на резистентност, който налага ефективно управление на антимикробните средства, редовно молекулярно наблюдение и засилени превантивни мерки в рамките на интегрираната рамка „Едно здраве“. Този подход би могъл да ограничи по-нататъшното разпространение на мултирезистентна *K. pneumoniae*, свързана с околната среда, клиничните фактори и общността.

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Introduction

Klebsiella pneumoniae is a Gram-negative, rod-shaped bacterium belonging to the *Enterobacteriaceae* family. *K. pneumoniae* is a common inhabitant of the intestinal tract of humans and warm-blooded animals and is ubiquitous in soil and freshwater aquatic habitats (Aldali, 2025). However, it has emerged as a significant opportunistic pathogen linked to various hospital-acquired infections, notably urinary tract infections (UTIs), wound infections, and life-threatening respiratory infections (Jin *et al.*, 2025). In recent years, *K. pneumoniae* has become clinically significant due to its rising role in nosocomial infections and its recognition as one of the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), a group of bacteria that are recognized by the World Health Organization (WHO) for their highly multidrug-resistant capacities and significant contribution to the global antimicrobial resistance crisis (Santajit and Indrawattana, 2016).

K. pneumoniae resistance to various antibiotics has been documented. This pathogen produces ESBL (extended-spectrum beta-lactamases) to resist virtually all beta-lactam antibiotics. Moreover, carbapenem-resistant strains of *K. pneumoniae* are frequently detected as well (Asri *et al.*, 2021; Jin *et al.*, 2025). *K. pneumoniae* also resists other antibiotics, including trimethoprim/sulfamethoxazole, aminoglycosides, tetracyclines, fluoroquinolones, and chloramphenicol (Asri *et al.*, 2021; Jin *et al.*, 2025). Infections of carbapenem-resistant or carbapenemase-producing *Enterobacteriaceae* have become a serious challenge in health-care settings. There has been a progressive increase in the global occurrence of carbapenem-resistant *K. pneumoniae* (CRKP) during the last decade (Karampatakis *et al.*, 2023).

CRKP strains are resistant to almost all available antimicrobials and often exhibit MDR (multi-drug resistance: resistance to one antibiotic from at least three antimicrobial classes), XDR (extensive drug resistance: resistance to at least one antibiotic from all but one antimicrobial class), and PDR (pan-drug resistance: resistance to all antibiotics in all antimicrobial classes) characteristics. These strains are associated with high mortality and morbidity rates, particularly in critically ill patients exposed to invasive devices (central venous catheters or ventilators) during prolonged hospitalization (Effah *et al.*, 2020; Asri *et al.*, 2021; Karampatakis

et al., 2023; Jin *et al.*, 2025).

K. pneumoniae infections have been documented in major cities (Makkah, Jeddah, and Riyadh) of Saudi Arabia (Alshahrani *et al.*, 2022; Hafiz *et al.*, 2023; Ibrahim, 2023; Jalal *et al.*, 2023; Aldali, 2025). Recent Saudi Arabian epidemiological studies have demonstrated an alarming rise in infections of MDR *K. pneumoniae* strains in clinical settings (Al-Zahrani and Alasiri, 2018; Al-Zalabani *et al.*, 2020; Booq *et al.*, 2022; Aldali, 2025; Idrees *et al.*, 2025). Due to the rising prevalence of MDR *K. pneumoniae* in Saudi Arabian health-care settings, continuous regional surveillance is crucial to understand the evolving resistance patterns of this pathogen. Thus, the current study aimed to examine the phenotypic and molecular resistance profiles of *K. pneumoniae* associated with UTIs, collected from three different hospitals in the Riyadh region of Saudi Arabia. We expected that these clinical isolates would demonstrate elevated rates of multidrug resistance, ESBL production, and genes encoding carbapenem resistance, indicating significant antimicrobial selective pressure in health-care facilities.

Materials and Methods

Sample collection

Klebsiella spp. isolates (26) were collected (November 2022 to January 2023) from Hospital (A) in Al-Kharj (4 isolates), Hospital (B) (18 isolates) in Riyadh, and Hospital (C) (4 isolates) in Riyadh, Saudi Arabia. All isolates were retrieved from male and female urinary tract infection (UTI) patients and identified as *Klebsiella* spp. The pure cultures of these isolates were transferred to the laboratory on sheep blood agar plates (Saudi Prepared Media Laboratory Company-SPML, Riyadh, Saudi Arabia) in an ice box in the dark. Microbiological examinations were initiated on the same day of receiving the isolates.

Identification of presumptive *Klebsiella* spp.

Isolates were initially collected from the hospitals as *Klebsiella* spp. Upon receipt in our laboratory, they were primarily identified by Gram staining, colonial morphology on agar plates (cysteine-lactose-electrolyte-deficient (CLED) and MacConkey) (Oxoid, UK), and API 20E strips (BioMérieux, France)-based biochemical tests (Osman *et al.*, 2020). The identity of isolates was further established through VITEK 2 Compact (BioMérieux, France) automated identification (Jalal *et al.*, 2023).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by adopting the automated VITEK 2 Com-

pact (BioMérieux) process (Jalal *et al.*, 2023). The minimum inhibitory concentrations (MICs) of 15 antibiotics were measured, which belonged to eight antimicrobial classes, including meropenem, imipenem [carbapenems], ampicillin [penicillins], gentamicin, amikacin [aminoglycosides], piperacillin/tazobactam and amoxicillin/clavulanic acid [beta lactam combination agents], ciprofloxacin [fluoroquinolones], ceftriaxone, cephalothin, cefoxitin, cefepime, ceftazidime, [cephems], trimethoprim/sulfamethoxazole [folate pathways antagonist], and nitrofurantoin [nitrofurans]. The results of susceptibility profiles were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2023)

Detection of antibiotic resistance genes

Genes encoding antibiotic resistance (*bla*_{KPC}, *bla*_{OXA-48}, *bla*_{TEM}, and *bla*_{CTX-M}) were detected using PCR utilizing previously described primers and amplification protocol (Ferreira *et al.*, 2019) (Table 1). The amplified products were visualized through ethidium bromide-containing agarose gel electrophoresis under UV light (Ferreira *et al.*, 2019).

Calculation of MARI (multiple antibiotic resistance index)

Multiple antibiotic resistance index (MARI) was calculated by dividing the number of antibiotics to which the isolate exhibited resistance by the total number of used antibiotics (Alami *et al.*, 2025). MARI value of more than 0.2 established the origin of the isolates from areas of frequent antibiotic usage. Contrarily, MARI < 0.2 demonstrated bacterial origin from areas of less antibiotic usage (Al-Ghamdi *et al.*, 2024).

16S rRNA gene sequencing and phylogenetic analysis

K. pneumoniae DNA was extracted using a DNeasy Blood and Tissue kit (QIAGEN, USA) in

accordance with the manufacturer’s instructions. DNA quality and quantity were assessed using spectrophotometry (Denovix DS-11, Denovix Inc., USA).

The 16s rRNA gene was amplified using universal primers 27F (forward: 5’ AGAGTTTGATC-MTGGCTCAG 3’) and 1492 R (reverse: 5’ TAC-GGYTACCTTGTTACGACTT 3’), in accordance with a previously described method (Khan *et al.*, 2024). PCR products were validated using agarose gel electrophoresis, purified using ExoSAP-IT™ kit (Thermo Fisher Scientific, USA), and subsequently analyzed via Sanger sequencing utilizing the SeqStudio platform (Life Technologies, USA).

The obtained sequences were analyzed using SNaGene (version 6.0.2, GSL Biotech LLC, USA), with the aid of the NCBI BLAST tool. Phylogenetic analysis was conducted using Geneious Prime® 2023.1.1 and MEGA 11.0 software. Sequence alignment was performed via MUSCLE, and a maximum parsimony phylogenetic tree was generated with 1000 bootstrap replications. *Escherichia coli* (accession number: J01859.1) served as the outgroup (Khan *et al.*, 2024)

Results

Antimicrobial susceptibility profiles of UTI-associated *K. pneumoniae*

All *K. pneumoniae* isolates (26) exhibited resistance to ampicillin [penicillins] (100%). Similarly, a significantly high resistance of 96% of isolates was noted against cepheems (cephalothin, ceftazidime, cefepime, ceftriaxone, and cefoxitin), fluoroquinolones (ciprofloxacin), and nitrofurantoin [nitrofurans]. Resistance to amoxicillin/clavulanic acid and piperacillin/tazobactam [beta-lactam combination agents] was observed in 89% and 85% of the *K. pneumoniae* isolates, respectively. 81% and 73% of the isolates were found to be resistant to meropenem and imipenem [carbapenems], respec-

Table 1. List of primers, amplicon size, and annealing temperatures

Target gene	Primer sequence	Annealing temperature (°C)	Amplicon Size (bp)	References
<i>bla</i> _{KPC}	F: 5'-CGTCTAGTTCTGCTGTCTTG-3' R: 5'-CTTGTCATCCTTGTTAGGCG-3'	61.3	797	Ferreira <i>et al.</i> (2019)
<i>bla</i> _{OXA-48}	F: 5'-GCGTGGTTAAGGATGAACAC-3' R: 5'-CATCAAGTTCAACCCAACCG-3'	55	438	Ferreira <i>et al.</i> (2019)
<i>bla</i> _{TEM}	F: 5'-TGCGGTATTATCCCGTGTTG-3' R: 5'-TCGTCGTTTGGTATGGCTTC-3'	63	296	Ferreira <i>et al.</i> (2019)
<i>bla</i> _{CTX-M}	F: 5'-ACAGCGATAACGTGGCGATG-3' R: 5'-TCGCCCAATGCTTTACCCAG-3'	64	216	Ferreira <i>et al.</i> (2019)

Table 2. Antibiotic susceptibility profiles of UTI-associated *K. pneumoniae*

Isolate	Antimicrobial agents															Resistance %
	AM	AMC	TZP	CET	FOX	CAZ	CRO	FEP	IMI	MEM	AK	GN	CIP	FM	SXT	
KP1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	94
KP2	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	94
KP3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP4	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	87
KP5	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S	87
KP6	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	94
KP7	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	94
KP8	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	80
KP9	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	94
KP10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP11	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	87
KP12	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP14	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP16	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	87
KP17	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	94
KP18	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	80
KP19	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP20	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	94
KP21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP22	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	100
KP23	R	R	S	R	S	R	R	R	S	S	S	S	R	R	R	60
KP24	R	S	S	R	R	R	R	R	S	S	S	R	R	R	R	67
KP25	R	S	S	R	R	R	R	R	S	S	S	R	R	R	R	67
KP26	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	7
Total	26	23	22	25	25	25	25	25	19	21	16	18	25	25	20	
(%)	(100)	(89)	(85)	(96)	(96)	(96)	(96)	(96)	(73)	(81)	(62)	(70)	(96)	(96)	(77)	

KP = *Klebsiella pneumoniae*, R = Resistant, S = Sensitive. AM = ampicillin, AMC = amoxicillin/clavulanic acid, TZP = piperacillin/tazobactam, CET = cephalothin, FOX = ceftiofur, CAZ = ceftazidime, CRO = ceftriaxone, FEP = cefepime, IMI = imipenem, MEM = meropenem, AK = amikacin, GN = gentamicin, CIP = ciprofloxacin, FM = nitrofurantoin, SXT = trimethoprim/sulfamethoxazole

tively. The resistance of *K. pneumoniae* isolates was comparatively lower against gentamicin (70%) and amikacin (62%) [aminoglycosides] (Table 2).

Most of the *K. pneumoniae* isolates (96%) from UTI samples were ESBL positive and exhibited multidrug resistance (MDR) (resistance to three or more antimicrobial classes). Notably, 20 isolates (77%) exhibited extensive drug resistance traits (XDR) (resistance to at least one antibiotic from all but one antimicrobial class) (Table 3), whereas one isolate (4%) was resistant to only one antibiotic (Table 3). The MARI values revealed that 25 isolates (96%) ranged between 0.6 and 1, which indicated their origin from environments with extensive antibiotic usage (Table 3). The majority of the *K. pneumoniae* isolates (96%) were phenotypically ESBL-positive (resistant to ceftazidime, cefepime, and ceftriaxone). Moreover, the detection of *bla*_{TEM}

genes confirmed genetically ESBL-positive isolates (89%), where one isolate (KP24) contained both *bla*_{TEM} and *bla*_{CTX-M} genes (Table 4). The data revealed that two phenotypically ESBL-positive isolates (KP23 and KP25) were genotypically ESBL-negative (Table 4). They might possess other beta-lactam resistance mechanisms like AmpC.

Hospital-based distribution of multidrug-resistant *K. pneumoniae* phenotypes depicted the presence of XDR (3) and MDR (1) isolates in Hospital A. Among hospital C-derived *K. pneumoniae*, all four isolates were XDR. Hospital B-derived *K. pneumoniae* isolates were distributed as XDR (13) and MDR (4) (Table 3).

Molecular characterization of antibiotic resistance genes in *K. pneumoniae*

The results revealed that 23 isolates (89%) were positive for the *bla*_{TEM} gene, whereas *bla*_{OXA-48}

Table 3. Multidrug-resistance patterns of UTI-associated *K. pneumoniae*

Isolate number	Isolate source	Resistance profile	Number of classes	Resistance pattern	ESBL	MARI
KP1	Hospital C	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM	7	XDR	+	0.93
KP2	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, AK, GN, CIP, FM, SXT	8	XDR	+	0.93
KP3	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP4	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, MEM, GN, CIP, FM, SXT	7	XDR	+	0.87
KP5	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, CIP, FM	7	XDR	+	0.87
KP6	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	0.93
KP7	Hospital A	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, CIP, FM, SXT	8	XDR	+	0.93
KP8	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, CIP, FM	6	MDR	+	0.8
KP9	Hospital A	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, CIP, FM, SXT	8	XDR	+	0.93
KP10	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP11	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, CIP, FM, SXT	7	XDR	+	0.87
KP12	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP13	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP14	Hospital C	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP15	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP16	Hospital A	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, GN, CIP, FM	7	XDR	+	0.87
KP17	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, GN, CIP, FM, SXT	8	XDR	+	0.93
KP18	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, CIP, FM	6	MDR	+	0.8
KP19	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP20	Hospital B	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	0.93
KP21	Hospital C	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP22	Hospital C	AM, AMC, TZP, CET, FOX, CAZ, CRO, FEP, IMI, MEM, AK, GN, CIP, FM, SXT	8	XDR	+	1
KP23	Hospital B	AM, AMC, CET, CAZ, CRO, FEP, CIP, FM, SXT	6	MDR	+	0.6
KP24	Hospital A	AM, CET, FOX, CAZ, CRO, FEP, GN, CIP, FM, SXT	6	MDR	+	0.67
KP25	Hospital B	AM, CET, FOX, CAZ, CRO, FEP, GN, CIP, FM, SXT	6	MDR	+	0.67
KP26	Hospital B	AM	1	-	-	0.07
Total number of ESBL isolates (%)				25 (96)		
Total number of MDR (%)				20 (77)		
Total number of XDR (%)						

AM = ampicillin, AMC = amoxicillin/clavulanic acid, TZP = piperacillin/tazobactam, CAZ = ceftazidime, FEP = cefepime, FOX = cefoxitin, CET = cephalothin, CRO = ceftriaxone, IMI = imipenem, MEM = meropenem, AK = amikacin, GN = gentamicin, CIP = ciprofloxacin, FM = nitrofurantoin, SXT = trimethoprim/sulfamethoxazole, ESBL = Extended-spectrum beta-lactamase.

MARI: Multiple Antibiotic Resistance Index, MDR: multidrug resistance, XDR: extensive drug resistance.

†MDR isolates counts also included XDR isolates.

‡Phenotypic criteria for ESBL inference: simultaneous resistance to third-generation cephalosporins (ceftazidime [CAZ], cefotaxime [CTX], and/or cefuroxime [CXM]), without resistance to carbapenems (ertapenem [ETP] and meropenem [MEM]).

and *bla*_{KPC} were detected in 11 (43%) and 10 (39%) isolates, respectively. Contrarily, the *bla*_{CTX-M} gene was carried by only one isolate (4%) (Table 4). Hospital-based diversity of antibiotic-resistance encoding genes indicated the occurrence of the *bla*_{TEM} gene in *K. pneumoniae* isolates in all three hospitals. However, *bla*_{KPC} and *bla*_{OXA-48} genes were predominantly detected in isolates from hospitals B and C. The *bla*_{CTX-M} gene-carrying isolate was collected from hospital A.

Phylogenetic analysis of *K. pneumoniae*

16S rRNA sequencing results confirmed the phenotypic identification of *K. pneumoniae* isolates. Phylogenetic analysis of the UTI-associated isolates distributed 26 isolates into three distinct clusters (Fig. 1).

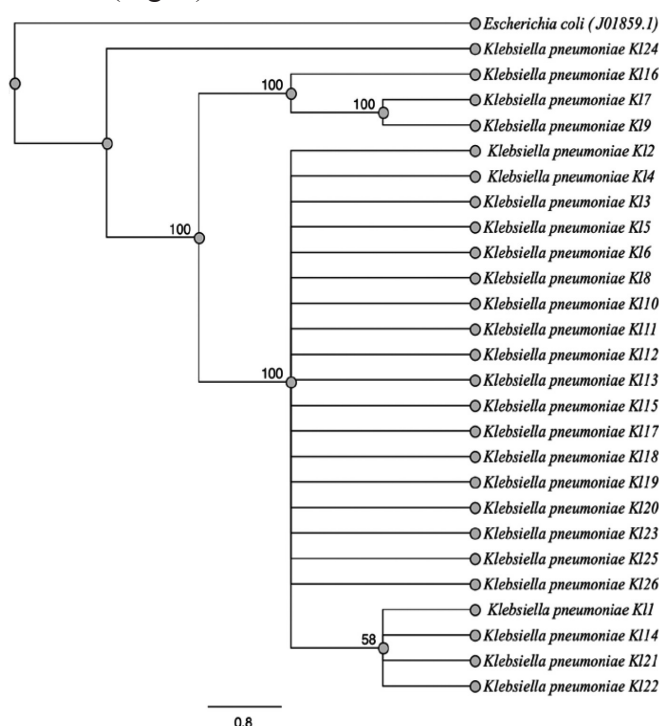


Fig. 1. 16S rRNA sequence-based phylogenetic comparison of *K. pneumoniae* isolates. Geneious Prime 2023 compared the sequences of all isolates. MUSCLE sequence alignment was followed by phylogenetic tree construction using the PAUP* plugin according to the maximum parsimony algorithm with 1000 replicates at each corresponding node. *Escherichia coli* (J01859.1) from the NCBI database served as the outgroup.

Eighteen isolates grouped and formed the largest cluster, whereas each of the other two clusters contained four isolates. Most clusters demonstrated a high level of confidence through strong bootstrap support (100%) in these phylogenetic groupings. Contrarily, one branch had comparatively lower support (58%), which suggested modest genetic divergence and reduced phylogenetic resolution within that subgroup. Overall, the phylogenetic tree represented high genetic similarity among

isolates, which is evident from close clustering and short branch lengths. Nevertheless, the cluster with lower bootstrap support indicates limited but noticeable diversity among a small subset of isolates.

Discussion

Klebsiella pneumoniae is a clinically important emerging opportunistic pathogen. Its resistance to numerous antibiotics has been documented with a rapid recent increase in MDR strains worldwide, including Saudi Arabia (Asri *et al.*, 2021; Jin *et al.*, 2025; Aldali, 2025). The current study examined UTI-related *K. pneumoniae* isolates (26), which were collected from three hospitals in the Riyadh region in central Saudi Arabia and studied for their antimicrobial susceptibility profiles.

The data revealed notable resistance of UTI-associated *K. pneumoniae* isolates against major antimicrobial classes, including nitrofurans, β -lactams, folate pathway antagonists, cephalosporins, fluoroquinolones, and carbapenems. The intrinsic characteristics of these isolates led to their complete resistance against ampicillin (100%) (Huy, 2024). The identified isolates also exhibited high resistance rates to third- and fourth-generation antibiotics [96% (nitrofurantoin, cephalosporins, and ciprofloxacin) and 77% (trimethoprim/sulfamethoxazole)], which raises serious public health concerns. Similar resistance trends of clinical *K. pneumoniae* isolates have been reported against these antimicrobial classes in Riyadh and other Saudi Arabian regions (Ibrahim, 2023; Hafiz *et al.*, 2023; Jalal *et al.*, 2023; Alshehri *et al.*, 2024; Aldali, 2025; Alharbi *et al.*, 2026). The rise in a wide range of resistance to various antimicrobial classes has been reported on a global scale as well (Asri *et al.*, 2021; Santella *et al.*, 2024; Song *et al.*, 2025). Differential resistance rates in local and international reports might be due to patient variation, antimicrobial usage policies in different clinical settings/ countries, and varying infection control measures in hospitals (Song *et al.*, 2025). Nevertheless, the results of this study suggest the ineffectiveness of several commonly used antimicrobials against *K. pneumoniae*-associated infections (Huy, 2024). These findings also denote sustained broad-spectrum antibiotic pressure and reduced empirical therapy options (Huy, 2024).

In recent years, the prevalence of carbapenem-resistant *K. pneumoniae* isolates has increased in various Saudi Arabian regions (Al-Zahrani *et al.*, 2018; Alshahrani *et al.*, 2022; Booq *et al.*, 2022; Hafiz *et al.*, 2023; Alfaifi *et al.*, 2024; Aldali, 2025;

Table 4. Diversity of antimicrobial resistance genes among UTI-associated *K. pneumoniae*

Isolate number	Isolate source	Resistance genes			
		<i>bla</i> _{KPC}	<i>bla</i> _{TEM}	<i>bla</i> _{CTX-M}	<i>bla</i> _{OXA-48}
KP1	Hospital C	-	+	-	-
KP2	Hospital B	-	+	-	-
KP3	Hospital B	-	+	-	+
KP4	Hospital B	+	+	-	-
KP5	Hospital B	+	+	-	+
KP6	Hospital B	-	+	-	+
KP7	Hospital A	-	+	-	-
KP8	Hospital B	-	+	-	-
KP9	Hospital A	-	+	-	+
KP10	Hospital B	-	+	-	-
KP11	Hospital B	+	+	-	+
KP12	Hospital B	+	+	-	-
KP13	Hospital B	+	+	-	-
KP14	Hospital C	+	+	-	+
KP15	Hospital B	-	+	-	-
KP16	Hospital A	-	+	-	-
KP17	Hospital B	+	+	-	-
KP18	Hospital B	-	+	-	+
KP19	Hospital B	+	+	-	+
KP20	Hospital B	+	+	-	+
KP21	Hospital C	+	+	-	+
KP22	Hospital C	-	+	-	+
KP23	Hospital B	-	-	-	-
KP24	Hospital A	-	+	+	-
KP25	Hospital B	-	-	-	-
KP26	Hospital B	-	-	-	-
Total (%)		10 (39)	23 (89)	1 (4)	11 (43)

Alharbi *et al.*, 2026). Similar trends have been reported in other countries as well (Krapp *et al.*, 2025; Lazar *et al.*, 2025; Qin *et al.*, 2025; Sisay *et al.*, 2025). Carbapenem-resistant *K. pneumoniae* (CRKP) is a particularly significant threat to global public health, as these antibiotics serve as the last defense line against MDR *Enterobacteriales*-associated severe infections (Huy, 2024; Aldali, 2025). CRKP-associated infections lead to high morbidity and mortality rates, particularly in ICU (intensive care units) and pneumonia patients. MGEs (mobile genetic elements) carry carbapenem-resistant genes, which rapidly spread between Gram-negative bacteria. This situation results in the development of carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) isolates causing severe infections in healthy individuals and immunocompromised patients (Karampatakis *et al.*, 2023). During

this study, higher carbapenem resistance was noted among UTI-associated *K. pneumoniae*, where most isolates were resistant to meropenem (81%) and imipenem (73 %). These findings are in line with previous Saudi Arabian and global reports (Idrees *et al.*, 2024; Aldali, 2025; Sisay *et al.*, 2025; Lazar *et al.*, 2025; Qin *et al.*, 2025; Krapp *et al.*, 2025; Shabi *et al.*, 2026). High carbapenem resistance rates in *K. pneumoniae* isolates were accompanied by high aminoglycoside resistance, suggesting their widespread circulation within the studied hospitals. It indicates rapid attenuation in the effectiveness of conventional treatments in healthcare settings (Asri *et al.*, 2021; Jin *et al.*, 2025; Shabi *et al.*, 2026).

The ESBL-producing *K. pneumoniae*, resisting common treatment options (penicillins and cephalosporins), pose a serious threat to healthcare settings and could lead to the emergence of MDR

strains (Idrees *et al.*, 2025). ESBL and ESBL-MDR *K. pneumoniae* infections are characterized by high mortality and morbidity rates (Asri *et al.*, 2021; Idrees *et al.*, 2025). A significant prevalence of phenotypic ESBL profile was observed in the current study, where almost all isolates (25/26) demonstrated resistance against third- and fourth-generation cephalosporins. It indicates frequent ESBL production among the *K. pneumoniae* isolates from all three studied hospitals. High rates of ESBL-producing *K. pneumoniae* are often reported in the Riyadh region of Saudi Arabia (Aldali, 2025). These reports are in agreement with the findings of the current study. However, the ESBL-producing *K. pneumoniae* rates could vary (higher or lower) in other Saudi Arabian regions due to different concentrations of administered antimicrobial agents and isolate detection time (Aldali, 2025). Most detected isolates (23 out of 26) carried the *bla*_{TEM} gene, whereas the *bla*_{CTX-M} gene was additionally detected in one isolate (KP24). These observations confirm the genetic foundation of ESBL synthesis in most isolates. The strong correlation between phenotypic resistance and identification of ESBL genes highlights TEM-type β -lactamases as the potential contributors to extended-spectrum cephalosporin resistance (Shaikh *et al.*, 2014). These findings necessitate phenotypic and molecular surveillance along with enhanced infection control protocols and antibiotic stewardship to restrict ESBL-producing *K. pneumoniae* dissemination in healthcare settings (Alfaifi *et al.*, 2024).

During the current study, the overall MDR prevalence was strikingly high (96%). A notable 77% of identified isolates were classified as XDR (extensively drug-resistant). MDR rates of 67% have been reported among Saudi Arabian clinical *K. pneumoniae* (Ibrahim, 2023). However, the occurrence of 96% MDR and 77% XDR rates among UTI-associated isolates is rare in Saudi Arabia (Balkhy *et al.*, 2020; Ibrahim, 2023). *K. pneumoniae* MDR rates vary in different regions of Saudi Arabia; however, an upward trend of MDR *K. pneumoniae* isolates has been observed in Saudi Arabian healthcare settings (Ibrahim, 2023; Aldali, 2025). The significantly high XDR rates of UTI-associated *K. pneumoniae* in the current study turn traditionally manageable infections into potential precursors of urosepsis-like, untreatable, and severe complications (Filev *et al.*, 2025).

MARI values of *K. pneumoniae* isolates further supported the MDR patterns observations. MARI values exceeded the 0.2 threshold for al-

most all the isolates, thus indicating their origin from high-risk environments with high antibiotic usage (Al-Ghamdi *et al.*, 2024). Such elevated values depicted continuous indiscriminate exposure to antibiotics within the hospital settings (Alami *et al.*, 2025). This pattern highlights the contribution of strong selective pressure in the development and persistence of highly resistant *K. pneumoniae* strains (Al-Ghamdi *et al.*, 2024).

The widespread detection of resistance-associated genes [*bla*_{TEM} (89%), *bla*_{OXA-48} (43%), and *bla*_{KPC} (39%)] closely aligned with the observation of carbapenem and β -lactam resistance phenotypes. Previous studies in the Riyadh region have reported frequent detection of *bla*_{TEM} and *bla*_{OXA-48} in clinical *K. pneumoniae*, which resembles the findings of this study. Contrarily, *bla*_{KPC} has been rarely reported in Riyadh previously. On the other hand, the genes of the *bla*_{CTX-M} family are commonly detected in resistant strains (Aldali, 2025). The *bla*_{CTX-M} gene has been the most predominant ESBL-resistance gene among *K. pneumoniae* isolates in various regions of Saudi Arabia, followed by *bla*_{TEM} and *bla*_{SHV}. Similarly, *bla*_{OXA-48} is a frequently prevalent carbapenem-resistant gene in Saudi Arabia (Idrees *et al.*, 2025; Shabi *et al.*, 2026). During the current study, the *bla*_{TEM} and *bla*_{OXA-48} prevalence were similar to other Arabian Peninsula (United Arab Emirates, Qatar, Oman, Kuwait, and Bahrain) and global reports (Asri *et al.*, 2021; Idrees *et al.*, 2025). Migration and international travel could have contributed to the ESBL-related gene spread and genetic diversity within Saudi Arabia and the Gulf region (Idrees *et al.*, 2025). The carbapenem-resistant *bla*_{OXA-48} gene is often detected in the Arabian Peninsula. However, the prevalence of *bla*_{KPC} varies in Arabian Peninsula nations, which could be due to the differential international travel environments and spreading pathways of carbapenem genes (Asri *et al.*, 2021; Idrees *et al.*, 2025). The higher prevalence of *bla*_{KPC} and *bla*_{OXA-48} in Hospitals B and C aligns with the XDR isolates' concentration in these facilities. Thus, it may support the gene-driven clonal expansion hypothesis. Contrarily, comparatively low *bla*_{CTX-M} detection may suggest TEM-type carbapenemase-oriented resistance rather than classical ESBL dissemination (Yassara *et al.*, 2025). The co-circulation of multiple carbapenemase genes could potentially facilitate interspecies spread and plasmid-mediated horizontal gene transfer (HGT) within the healthcare environments (Koh *et al.*, 2025).

According to the One Health perspective,

these observations highlight a broader problem that expands beyond hospital settings. Carbapenemase-producing *K. pneumoniae* isolates serve as mobile genetic reservoirs, which can spread across clinical, agricultural, and environmental interfaces (Huang *et al.*, 2025; Jin *et al.*, 2025). Hospitals under intense antimicrobial pressure might amplify environmental persistence and release of resistant strains into wastewater channels (Samreen *et al.*, 2021; Jin *et al.*, 2025; Martinez-Ruiz *et al.*, 2026). Such ecological circulation promotes their communal reintroduction, thereby broadening resistance reservoirs (Huang *et al.*, 2025; Jin *et al.*, 2025).

Limitation of the study

There are certain limitations of this study. The limited sample size (26 isolates from three hospitals in Riyadh) might hinder the generalizability of the findings to other Saudi Arabian healthcare environments. Furthermore, the brief sampling duration might have inadequately represented potential temporal fluctuations in antibiotic resistance trends. The molecular screening focused on specific resistance genes (*bla*_{TEM}, *bla*_{KPC}, *bla*_{OXA-48}, and *bla*_{CTX-M}). Therefore, other mobile genetic elements or resistance determinants might have remained undetected. The lack of high-resolution genomic investigations (multilocus sequence typing and whole-genome sequencing) during the study could have restricted the exploration of clonal relatedness, transmission channels, and the entirety of isolates' resistome. Despite these limitations, the study provides significant early evidence of multidrug- and extensively drug-resistant *K. pneumoniae* prevalence in examined hospitals.

Conclusion

The current study reveals an alarmingly high burden of antimicrobial-resistant UTI-associated *K. pneumoniae* isolates in the studied hospitals in the Riyadh region. Approximately all isolates (96%) were found to be multidrug-resistant, whereas 77% were classified as extensively drug-resistant isolates. The widespread occurrence of *bla*_{TEM}, along with significant detection of *bla*_{KPC} and *bla*_{OXA-48}, confirmed β -lactam and carbapenem resistance at the molecular level. These results indicated active dissemination of carbapenemase-producing strains. The prevalence of highly resistant isolates combined with elevated MARI values (> 0.6), particularly in Hospital B, demonstrated intense antimicrobial selection pressure and sustained clonal transmission within healthcare settings. Collectively, these results denote a critical resistance thresh-

old and demand effective antimicrobial stewardship, regular molecular surveillance, and improved infection-preventing measures under an integrated One Health framework. These steps could restrict further environmental, clinical, and communal spreading of multidrug-resistant *K. pneumoniae*.

Institutional Review Board Statement

This study was approved by the research ethics committee of the Department of Biology, College of Science (Approval # 342080714439/67979) on 17 May 2022. The study does not include human and/or animal subjects.

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