Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2025;50(4):1153-1162
DOI: 10.17826/cumj.1781009

RESEARCH

Investigation of carbapenemase and ESBL status, capsule serotypes, and clinical distribution of virulence genes in *Klebsiella pneumoniae* isolates

Klebsiella pneumoniae izolatlarında karbapenemaz ve ESBL durumu ile kapsül serotipleri ve virulans genlerinin klinik dağılımının incelenmesi

Başak Baykara[1], Hamdullah Suphi Bayraktar[2], Fariz Selimli[2]

[1]Hakkari University, Hakkari, Türkiye
[2]Hatay Mustafa Kemal University, Hatay, Türkiye

Abstract

Purpose: This study aimed to (i) determine the prevalence of capsule serotypes and virulence genes in *Klebsiella pneumoniae* clinical isolates obtained from the Çukurova region of Turkey, and (ii) assess their relationship with carbapenemase and extended-spectrum β-lactamase (ESBL) production.

Materials and Methods: A total of 353 *K. pneumoniae* isolates were recovered from various clinical specimens of both inpatients and outpatients. Carbapenem susceptibility and ESBL production were initially evaluated using an automated antimicrobial susceptibility testing system and subsequently confirmed by the Kirby–Bauer disc diffusion assay and the combined disc method, following EUCAST 2025 guidelines. Genomic DNA was extracted from overnight cultures, and species identification was verified using PCR targeting the 16S–23S ITS region. Capsule serotypes and virulence-associated genes were detected through specific PCR-based assays.

Results: Carbapenemase and ESBL production were identified in 26.1% (n = 92) and 23.2% (n = 82) of isolates, respectively, with 17.3% (n = 61) co-producing both enzymes. Capsule serotypes were detected in 11.6% of isolates, predominantly K2 (5.1%, n = 18) and K5 (6.5%, n = 23), whereas K1 was not found. The frequencies of virulence genes were as follows: *mrkD* (90%), *fimH-1* (80%), *ybtS* (92%), *iutA* (78%), *rmpA* (10%), and *iroN* (2%). A statistically significant association was observed between antimicrobial resistance phenotypes and selected virulence genes, including *mrkD*, *ybtS*, and *iutA*.

Conclusion: The findings reveal the distribution of capsule serotypes and virulence determinants among *K. pneumoniae* isolates circulating in the Çukurova region. These results highlight the importance of sustained

Öz

Amaç: Bu çalışmanın iki temel amacı vardır: Birincisi, Türkiye'nin Çukurova bölgesindeki hastanelerde *Klebsiella pneumoniae* enfeksiyonlarında kapsüler serotipler ve virülans genlerinin prevalansını araştırmak; ikinci olarak karbapenemaz ve ESBL üretimi arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Toplam 353 *K. pneumoniae* klinik izolatı yatan ve ayaktan hastaların çeşitli klinik örneklerinden elde edilmiştir. Karbapenem duyarlılığı ve ESBL üretimi, otomatik antimikrobiyal duyarlılık test sistemi kullanılarak ve Kirby–Bauer disk difüzyon yöntemi ile kombinasyon disk testi ile doğrulanarak EUCAST 2025 kılavuzlarına uygun şekilde belirlenmiştir. Genomik DNA, gece boyunca kültürlerden ekstrakte edilmiş ve 16S–23S ITS bölgesine yönelik PCR ile *K. pneumoniae* varlığı doğrulanmıştır. Kapsüler serotipleri ve virülans genleri, spesifik PCR tabanlı testlerle saptanmıştır.

Bulgular: *K. pneumoniae* izolatlarının %26,1'inde (n=92) karbapenemaz ve %23,2'sinde (n=82) ESBL üretimi saptanmış olup, her ikisi de izolatların %17,3'ünde (n=61) görülmüştür. Kapsüler serotipler izolatların %11,6'sında tespit edilmiş, en sık K2 (%5,1, n=18) ve K5 (%6,5, n=23) gözlenmiş; K1 saptanmamıştır. Virülans genlerinin prevalansı ise *mrkD* %90, *fimH-1* %80, *ybtS* %92, *iutA* %78, *rmpA* %10 ve *iroN* %2 olarak bulunmuştur. Direnç fenotipleri ile *mrkD, ybtS* ve *iutA* arasında istatistiksel olarak anlamlı bir ilişki saptanmış; bu virülans genleri karbapenemaz/ESBL üretimi ile birlikte görülmüştür.

Sonuç: Çalışma, Çukurova bölgesindeki *K. pneumoniae* izolatlarında kapsüler serotipler ve virülans genlerinin dağılımını ortaya koyarak, enfeksiyonların önlenmesi ve akılcı antimikrobiyal tedavi açısından sürekli moleküler izlemenin önemini vurgulamaktadır.

Address for Correspondence: Hamdullah Suphi Bayraktar, Hatay Mustafa Kemal University, Experimental Researches Application and Research Center; Hatay, Türkiye E-mail: drsuphibayraktar@gmail.com
Received: 10.09.2025 Accepted: 17.12.2025

molecular surveillance to inform infection control strategies and optimize antimicrobial stewardship.
Keywords:. Capsule serotypes, carbapenem resistance, ESBL, hospital-acquired infections, *Klebsiella pneumoniae*, virulence genes.

Anahtar kelimeler: ESBL, hastane kaynaklı enfeksiyonlar, karbapenem direnci, kapsüler serotipler, *Klebsiella pneumoniae*, virülans genleri

INTRODUCTION

Klebsiella pneumoniae is one of the leading Gram-negative opportunistic pathogens responsible for a wide spectrum of community-acquired and healthcare-associated infections, including urinary tract infections, pneumonia, bloodstream infections, and surgical-site infections[1,2]. These infections are associated with substantial morbidity and mortality, particularly among critically ill patients, individuals with malignancies, the elderly, and those exposed to invasive medical devices[3,4]. In parallel with the global rise in antimicrobial resistance, the rapid expansion of extended-spectrum β-lactamase (ESBL)-producing and carbapenemase-producing *K. pneumoniae* has emerged as a major public health threat, severely restricting treatment options and increasing the burden on healthcare systems[5,6,7].

The pathogenicity of *K. pneumoniae* is driven by a complex arsenal of virulence factors, among which the capsular polysaccharide is considered to be the most critical determinant[8,9,10]. The capsule is resistant to serum bactericidal activity and phagocytosis, and it plays a central role in immune evasion. Certain capsular serotypes, including K1, K2, K5, K20, K54, and K57, are closely associated with severe infections, septicemia, liver abscesses, and generally poor patient prognoses[11,12,13]. A comprehensive genetic study of bacterial samples taken from the bloodstream, conducted by Khrulnova et al., revealed that particularly aggressive *K. pneumoniae* strains were significantly more prevalent among those resistant to carbapenem antibiotics. ST23/K57 and ST395/K2 were identified as dominant high-risk clones among patients with hematological malignancies. These findings emphasize the growing importance of addressing carbapenem-resistant, highly virulent *K. pneumoniae* in clinical settings[13,14,15,16].

In addition to capsular characteristics, *K. pneumoniae* relies heavily on adhesion and iron-acquisition systems, which are key determinants of its persistence in hospital environments[9,17]. Adhesins such as *fimH-1* and *mrkD* mediate attachment to uroepithelial and respiratory epithelial cells[18,19]. These proteins also promote biofilm formation on medical equipment used inside the body[20]. Siderophore systems encoded by *ybtS, iutA*, and *iroN* provide efficient iron acquisition in the iron-limited host milieu, and they significantly enhance bacterial survival and dissemination[1,17]. Virulence is further enhanced by hypermucoviscosity regulators, especially *rmpA* and *rmpA2*, which promote invasive phenotypes and increase capsule production[8,21].

Classical multidrug-resistant (MDR) *K. pneumoniae* and hypervirulent *K. pneumoniae* used to be considered distinct populations. However, this paradigm has shifted dramatically in recent years due to the convergence of resistance and virulence[5,16,22]. Horizontal gene transfer via plasmids facilitates the dissemination of genetic material between bacterial populations[23,24,25]. These plasmids may harbor both carbapenem resistance genes and virulence determinants, leading to the emergence of highly virulent and carbapenem-resistant *K. pneumoniae* strains[16,26]. These organisms pose a major therapeutic and epidemiological challenge for both clinical management and public health control[27,28]. Soltani et al. stated that there was a significant association between carbapenemase genes and specific capsular serotypes, particularly K20 and K54, and demonstrated widespread MDR and ESBL production among clinical *K. pneumoniae* isolates in a hospital setting. Their findings highlight how serotype-specific resistance patterns may contribute to endemic transmission[6,29,30].

Similarly, Ilyas et al. reported an alarmingly high prevalence of MDR *K. pneumoniae* (over 90%), accompanied by extensive virulence traits, including strong biofilm formation, efflux pump activity, outer membrane porin alterations, and hypermucoviscosity. The researchers identified a strong correlation between MDR and virulence-associated phenotypes, particularly biofilm production ($r = 0.99$), efflux pumps ($r = 0.92$), and porin loss ($r = 0.88$), demonstrating that resistance and pathogenicity frequently evolve concurrently under hospital selective pressure[5,9,17].

The epidemiology of capsular serotypes, virulence gene profiles, and resistance mechanisms varies

considerably between geographic regions, reflecting differences in antibiotic consumption, clonal dissemination, infection-control practices, and plasmid circulation[1,16,22,23]. While several international studies have described the molecular landscape of hypervirulent and carbapenem-resistant *K. pneumoniae*[8,10,12,21], comprehensive regional data from southern Turkey—particularly the Çukurova region—are still lacking. This gap makes it difficult to monitor the region effectively and to classify risks for hospital departments with high burdens such as intensive care units and urology services[2,4,7].

This study represents a comprehensive molecular investigation into the capsular serotypes and virulence factors of carbapenemase- and ESBL-producing *K. pneumoniae* isolated in the Çukurova region. It also provides important local information that can assist in creating specific strategies for infection control and surveillance, as well as guiding early empirical antibiotic therapy[6,29,30].

Therefore, the present study aimed to characterize the distribution of key capsular serotypes (K1, K2, and K5) and major virulence genes (*mrkD, fimH-1, ybtS, iroN, iutA,* and *rmpA*) among clinical *K. pneumoniae* isolates recovered from hospitals in the Çukurova region of Turkey, and to evaluate their association with carbapenemase and ESBL production[12,14,20]. By integrating phenotypic antimicrobial resistance profiling with targeted molecular detection of virulence and capsule determinants, this study also provides a comprehensive regional snapshot of virulence–resistance convergence and evidence-based infection control and antimicrobial stewardship strategies[29,30,31].

MATERIALS AND METHODS

This retrospective observational study was carried out between August 2023 and January 2025 at hospitals across the Çukurova region, Türkiye. Ethical approval was obtained from the Ethical Committee for Clinical Research of Mustafa Kemal University (Decision No: 11/45, Meeting Date: 27.08.2025). Informed consent was not required as only residual clinical samples were used.

Clinical isolates

Clinical specimens were collected from patients presenting with signs of infection using standard aseptic techniques. Blood cultures or other specimens showing growth of common skin flora without clinical evidence of infection were excluded. Repeated isolates from the same patient were excluded, and only one isolate per patient was included in the study. A total of 353 *K. pneumoniae* isolates were collected from urine, tracheal aspirates, blood, wound swabs, sputum, and catheter samples, originating from various hospital units including intensive care units and outpatient clinics. Detailed specimen distributions are presented in the Results section.

Antibiotic susceptibility testing

Carbapenem susceptibility and ESBL production were determined using the VITEK® 2 Compact automated system (bioMérieux, France) following EUCAST 2025 guidelines. Carbapenem susceptibility was confirmed by the Kirby–Bauer disk diffusion method, and ESBL production was further verified with the combined disk test. *K. pneumoniae* ATCC 700603 was used as a quality control strain for ESBL detection, in accordance with EUCAST recommendations.

Genomic DNA extraction and PCR

Genomic DNA was extracted from overnight cultures in Luria Broth. The quality and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific) and integrity was verified on 1% agarose gel. PCR targeting the 16S–23S ITS region was used to confirm *K. pneumoniae* isolates. Specific PCR assays were conducted to detect capsular serotypes (K1, K2, K5) and virulence genes (*mrkD, fimH-1, ybtS, iroN, iutA, rmpA*). PCR products were assessed on 2% agarose gels. Primer sequences and detailed PCR conditions are provided in Tables 1–3.

Table 1. Primer sequences targeting the 16S–23S ITS region of *K. pneumoniae* [32]

Primer	Sequence (5'→3')	Amplicon size (bp)
K. pneumoniae F	ATTTGAAGAGGTTGCAAACGAT	
K. pneumoniae R	TTCACTCTGAAGTTTTCTTGTGTTC	130

ITS: Internal Transcribed Spacer; bp: base pairs; F: forward primer; R: reverse primer.

The primers in Table 1 were used for species-level confirmation of *K. pneumoniae* isolates through amplification of the 16S–23S ITS region (Table 1). The listed primers and annealing temperatures were used to identify K1, K2, and K5 capsular serotypes in the isolates (Table 2). The primer sets in Table 3 were used for the detection of key virulence genes associated with adhesion, iron acquisition, and hypervirulence in *K. pneumonia* (Table 3).

Table 2. Primer sequences for capsular serotype identification of *K. pneumoniae* [12]

Capsule Type	Primer	Sequence (5'→3')	Annealing (°C)	Amplicon size (bp)
K1	MagA-F	GGTGCTCTTTACATCATTGC	57	1283
K1	MagA-R	GCAATGGCCATTTGCGTTAG	57	1283
K2	K2wzy-F	GACCCGATATTCATACTTGACAGAG	57	641
K2	K2wzt-R	CCTGAAGTAAAATCGTAAATAGATGGC	57	641
K5	K5wzx-F	TGGTAGTGATGCTCGCGA	55	280
K5	K5wzx-R	CCTGAACCCACCCCAATC	55	280

K1, K2, K5: Capsular serotypes; F: forward primer; R: reverse primer; °C: degree Celsius; bp: base pairs.

Table 3. Primer sequences for virulence gene detection in *K. pneumoniae* [5]

Virulence Gene	Primer	Sequence (5'→3')	Annealing (°C)	Amplicon size (bp)
fimH-1	F	ATGAACGCCTGGTCCTTTGC	63	688
fimH-1	R	GCTGAACGCCTATCCCCTGC	63	688
mrkD	F	CCACCAACTATTCCCTCGAA	52	226
mrkD	R	ATGGAACCCACATCGACATT	52	226
iroN	F	AAGTCAAAGCAGGGGTTGCCCG	52	665
iroN	R	GACGCCGACATTAAGACGCAG	52	665
iutA	F	GGCTGGACATCATGGGAACTGG	52	300
iutA	R	CGTCGGGAACGGGTAGAATCG	52	300
ybtS	F	AGTGGTGCGTTCTGCGTC	52	477
ybtS	R	ATTTCTACATCTGGCGTTA	52	477
rmpA	F	ACTGGGCTACCTCTGCTTCA	52	535
rmpA	R	CTTGCATGAGCCATCTTTCA	52	535

fimH-1: type 1 fimbrial adhesin gene; *mrkD*: type 3 fimbrial adhesin gene; *iroN*: salmochelin siderophore receptor gene; *iutA*: aerobactin siderophore receptor gene; *ybtS*: yersiniabactin biosynthesis gene; *rmpA*: regulator of mucoid phenotype A; F: forward primer; R: reverse primer; °C: degree Celsius; bp: base pairs.

Statistical analysis

Categorical variables including age, gender, specimen type, capsular serotype, virulence gene positivity, and antibiotic resistance were expressed as frequencies and percentages. Associations between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. Correlations between virulence gene positivity and antibiotic resistance were evaluated using Spearman's rank correlation coefficient. Statistical analyses were performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

RESULTS

Of the 353 patients, 200 (56.7%) were male and 153 (43.3%) were female. Pediatric patients (0–18 years) were 57.9% male, adults (19–64 years) 60.6% male, and elderly patients (≥65 years) had nearly equal gender distribution. No significant association was observed between age and gender ($\chi^2 = 1.47$, $p = 0.20$; Figure 1). This reflects a balanced gender distribution across age groups. Figure 1 shows the distribution of patients across different age groups and indicates that males and females are represented in similar proportions among children, adults, and elderly patients.

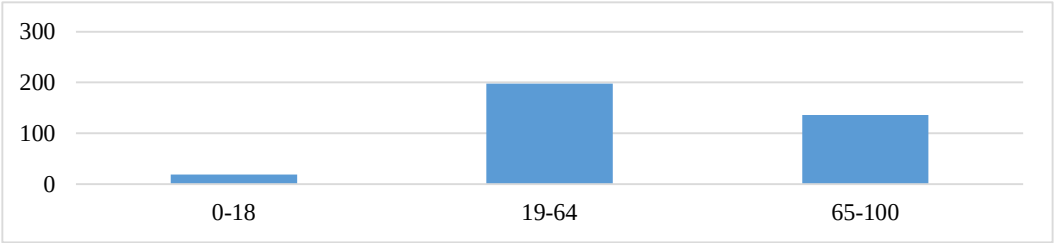


Figure 1. Age group distribution of the patients

Urine accounted for the majority of isolates (91%). Tracheal aspirates and blood samples were predominantly obtained from ICU patients. A significant association was observed between specimen type and clinical department ($\chi^2 = 18.6$, $p = 0.018$). The highest number of isolates originated from the Urology Outpatient Clinic (19.6%) and the Anesthesia & Reanimation ICU (11.3%).

Among the isolates, 26.1% were positive for carbapenemase only, 23.2% for ESBL only, 17.3% for both, and 33.4% were susceptible. Resistance rates were higher among male patients ($\chi^2 = 5.41$, $p = 0.02$) and increased with age. Carbapenemase positivity was highest in ICUs (64%) and urology service (42–48%), whereas ESBL positivity predominated in pediatric and obstetrics/gynecology service (Table 4).

Table 4. Resistance profiles by clinical services and age groups

Unit / Service	Age Group	ESBL+ Only	KPE+ Only	ESBL+ KPE+	Susceptible	Total
Urology Outpatient Clinic	Adult	10	12	9	38	69
Anesthesia & Reanimation ICU	Elderly	9	15	12	4	40
Internal Medicine ICU	Elderly	15	10	8	4	37
Internal Medicine Nephrology	Elderly	10	12	6	4	32
Surgical ICU	Adult	10	9	7	5	31
Obstetrics & Gynecology Outpatient Clinic	Adult	2	1	0	22	25
Infectious Diseases Outpatient Clinic	Adult	7	6	5	6	24
Urology Service	Adult	6	7	5	0	18
General Surgery Service	Adult	3	4	3	3	13
Internal Medicine General Ward	Elderly	3	4	2	1	10
Neurosurgery ICU	Child	1	1	1	7	10
Orthopedics & Traumatology	Adult	2	3	1	2	8
Coronary ICU	Elderly	2	2	1	1	6
Pediatrics Outpatient Clinic	Child	0	0	1	5	6
Internal Medicine Gastroenterology	Elderly	1	2	1	0	4
Internal Medicine Hematology	Elderly	1	1	0	1	3
Cardiology ICU	Elderly	1	1	0	1	3
Endocrinology Outpatient Clinic	Elderly	1	1	0	1	3
Neurology ICU	Elderly	1	1	1	0	3
Internal Medicine Rheumatology	Elderly	0	1	0	1	2
Dermatology Outpatient Clinic	Adult	0	1	0	1	2
Pulmonology Outpatient Clinic	Elderly	0	1	0	1	2
Cardiovascular Surgery Service	Elderly	0	0	0	1	1
Family Medicine	Elderly	0	0	0	1	1

ESBL: Extended-Spectrum β-Lactamase; KPE: Carbapenemase-Producing Enterobacteriaceae; ICU: Intensive Care Unit; +: positive; Child: 0–18 years; Adult: 19–64 years; Elderly: ≥65 years.
Due to small sample sizes in certain subgroups, these results should be interpreted with caution

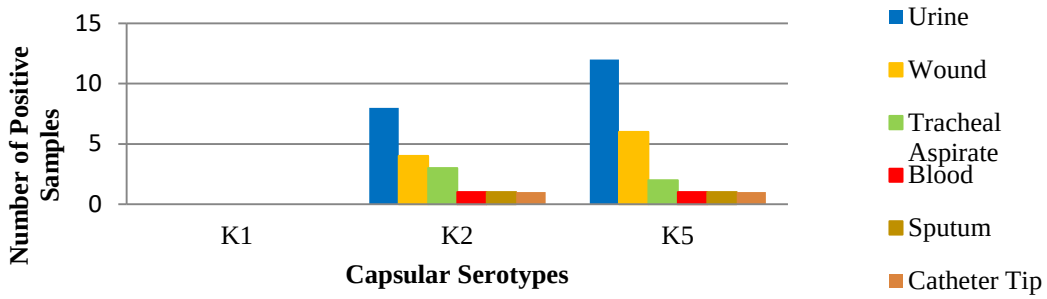


Figure 2. Capsular serotypes by specimen type

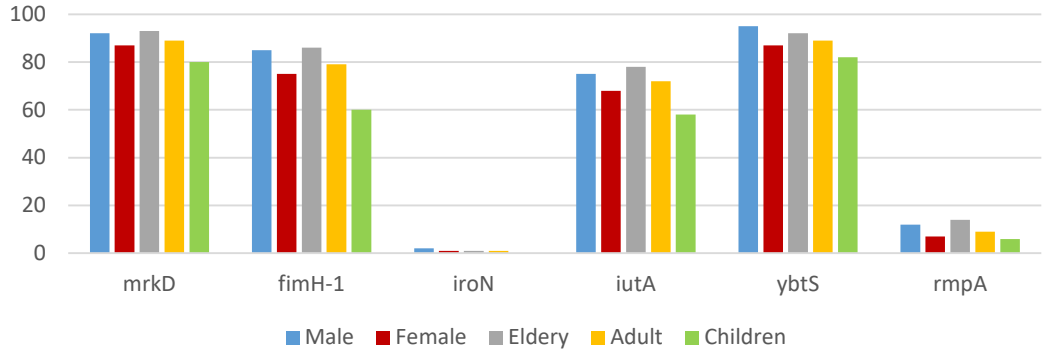


Figure 3. Virulence gene positivity rates: distribution by gender and age groups.

Table 4 summarizes the antibiotic resistance profiles of *K. pneumoniae* isolates obtained from different hospital units and age groups. As shown in the table carbapenemase-producing isolates were more common in intensive care and urology service, whereas ESBL producers predominated in pediatric and obstetrics/gynecology wards. When examined as a whole, resistance rates were higher in male patients and tended to increase with patient age. It is suggested that targeted infection control and antimicrobial stewardship efforts may be particularly important.

All isolates were confirmed as *K. pneumoniae* by PCR. Capsular serotyping revealed K2 in 5.1% and K5 in 6.5%; K1 was not detected. Capsule positivity was higher in adults and elderly patients and most prevalent in ICUs. The overall prevalence of virulence genes was as follows: *mrkD* 90%, *fimH-1* 80%, *ybtS* 92%, *iroN* 2%, *iutA* 78%, and *rmpA* 10%. Higher prevalence was observed in adult and elderly ICU and urology patients. Urine samples had the highest virulence gene positivity, whereas wound samples showed elevated *rmpA* (15%) (Figure 2). Figure 2 highlights the higher prevalence of K2 and K5 capsular serotypes in urine samples and ICU patients.

Virulence gene positivity was determined among the groups and *iroN* was found to be the rarest gene in *K. pneumoniae* isolates (Figure 3). Figure 3 illustrates the distribution of virulence gene positivity stratified by gender and age, highlighting differences across patient subgroups.

Both K2 and K5 serotypes exhibited high virulence gene prevalence and multidrug resistance. K2 strains showed 33% carbapenemase and 28% ESBL positivity; K5 strains showed 57% and 52%, respectively. No significant association was observed between capsular serotype and antibiotic resistance ($p > 0.05$), which might reflect the limited sample size rather than the absence of a true relationship. Carbapenemase- and ESBL-positive isolates showed higher virulence gene positivity than negative strains ($r = 0.88–0.99$; Table 5).

Table 5. Relationship between capsular serotype, virulence genes, and antibiotic resistance profile

Capsular Serotype	Virulence Gene Positivity (%)	Antibiotic Resistance Profile (Carbapenemase / ESBL, %)	Correlation with Resistance (r)	Overall Assessment
K2	85–90	Carbapenemase 33%, ESBL 28%	0.88–0.99	High virulence and moderate–high resistance; no statistically significant association between serotype and resistance ($p > 0.05$)
K5	80–85	Carbapenemase 57%, ESBL 52%	0.88–0.99	High virulence and high resistance; no statistically significant association between serotype and resistance ($p > 0.05$)

Findings for certain capsular serotype subgroups are based on limited sample sizes and should be interpreted with caution.
K2, K5: capsular serotypes; ESBL: Extended-Spectrum β-Lactamase; r: Spearman correlation coefficient; %: percentage.

Table 5 highlights the frequent co-occurrence of multidrug resistance and virulence genes, summarizes virulence gene positivity and antibiotic resistance profiles for K2 and K5 capsular serotypes.

Overall, these findings indicate that MDR and virulence genes frequently co-occur, and caution is needed when interpreting data from small subgroups.

DISCUSSION

High correlation coefficients between MDR and virulence genes (r = 0.88–0.99) suggest a co-occurrence of hypervirulence and antibiotic resistance, highlighting the need for early detection and targeted intervention in ICU settings. *K. pneumoniae* is a Gram-negative pathogen that poses a significant clinical challenge, particularly in hospital-acquired infections[33]. Its capsule is a key virulence factor, enabling resistance to serum bactericidal agents and evasion of phagocytosis. Structural variations in capsular polysaccharides influence the bacterium's association with different infection types, with some serotypes exhibiting high virulence and others being less pathogenic[10,11,34].

Capsular serotypes are often linked to antibiotic resistance and invasiveness. K2 and K5 serotypes have frequently been associated with sepsis, systemic infections, and liver abscesses, with K5 demonstrating particularly high invasiveness and multidrug resistance. In our study, 5.1% (18 of 353 isolates) were found to harbor K2 and 6.5% (23 of 353 isolates) contained K5, but no K1 was detected. This observation highlights the influence of regional and clonal variations of capsular distribution and provides an indirect argument for a rigorous surveillance system, especially because of the high K1 prevalence reported worldwide[35].

Hybrid plasmids carrying both carbapenemase genes (e.g., blaNDM, blaOXA-48) and virulence factors—particularly siderophore systems—have been documented in high-risk lineages. These plasmid-mediated mechanisms likely contribute to the high MDR–virulence correlation observed in our isolates[36]. It has been reported that IncHI1B (pNDM-MAR)/IncFIB(pNDM-MAR)-like plasmids frequently harbor resistance and virulence genes simultaneously, facilitating their co-dissemination. It was demonstrated that co-conjugation of virulence plasmids and blaKPC-2–bearing plasmids can convert classical *K. pneumoniae* strains into carbapenem-resistant hypervirulent *K. pneumoniae* (CR-HvKP), further accelerating the spread of MDR-HvKP strains in clinical settings. It was also shown that fusion of conjugative virulence plasmids (IncHI1B/repB) with KPC-type resistance plasmids generates highly transferable hybrid plasmids that co-harbor blaKPC and virulence determinants, promoting rapid dissemination of CR-HvKP. These observations are consistent with studies highlighting the central role of plasmids in the emergence, dissemination, and co-evolution of resistance and virulence in CR-*K. pneumoniae*. Moreover, even when CR-HvKP strains carrying hypervirulence plasmids are in vitro susceptible to ceftazidime/avibactam-based therapies, transcriptomic analyses reveal tolerance mechanisms involving altered cell respiration, carbohydrate transport, and stress-response pathways. Accordingly, the strong MDR–virulence correlation observed in our isolates may similarly reflect plasmid-mediated gene transfer,

emphasizing the need for continuous plasmid surveillance.

Regarding the virulence genes in the present study, *mrkD, fimH-1, ybtS*, and *iutA* were detected more frequently in adult patients hospitalized in the ICUs and urology service. Capsule-positive isolates were mainly observed in patients aged 50–55 years, which may be associated with early immunosenescence and increased hospital exposure in this age group[35]. In addition, the adherence genes *mrkD* and *fimH-1* were linked to biofilm formation, highlighting the need for targeted infection-control strategies to prevent catheter-associated device infections[33,34].

No statistically significant association was found between antibiotic resistance and capsular serotype. However, both K2 and K5 isolates showed a high prevalence of virulence genes and multidrug resistance, which may have important clinical implications[33,35]. Specifically, K5 isolates exhibited greater resistance to cephalosporins and carbapenems than K2 isolates. The strong correlations between MDR and virulence genes (Spearman r = 0.88–0.99) suggest that hypervirulence and multidrug resistance are likely being selected concurrently, which supports the need for early detection and individualized treatment in ICU settings. These results are consistent with previous reports[5,7,15].

The findings of regional studies have revealed wide local variation in capsular serotype and virulence gene distribution[33,37]. In Ankara, K1 was detected in 34% and K2 in 4.4% of the strains, whereas a study from southeastern Turkey reported predominantly non-typable isolates[14,15]. These findings underscore the importance of sustained regional surveillance to detect hypervirulent, multidrug-resistant strains and to inform the development of infection-control strategies.

This study offers a regional perspective on the relationships between *K. pneumoniae* capsular serotypes, virulence genes, and antibiotic resistance. Our results suggest that K2 and K5 isolates demonstrated both hypervirulence and multidrug resistance, while certain circulating clones maintain virulence alongside antimicrobial resistance.

From a clinical standpoint, our findings support screening, early isolation, and appropriate antibiotic therapy, particularly in high-risk populations such as ICU and transplant recipients. Surveillance of capsular serotypes and virulence gene profiles may allow timely identification of potential MDR-HvKP outbreaks[35,36].

This study has several limitations. Detailed clinical data, including comorbidities, prior antibiotic use, and hospitalization history, were unavailable, which may limit accurate risk stratification[2,3,28]. Antimicrobial susceptibility testing was restricted to carbapenemase and ESBL production, and other antibiotic classes were not tested in all isolates, potentially limiting the comprehensiveness of the resistance profile. In addition, community-acquired and hospital-acquired infections were not differentiated. Molecular analysis was also limited to targeted PCR assays; whole-genome sequencing and detailed plasmid analyses were not performed, restricting insights into clonal relatedness and transmission dynamics[7,16,23,24]. Finally, the study was conducted in a single region, which may limit generalizability[29].

Despite these limitations, the study provides important regional insight into the convergence of multidrug resistance and virulence in *K. pneumoniae*, particularly among K2 and K5 serotypes. Future studies prioritizing multicenter, longitudinal surveillance incorporating whole-genome sequencing and plasmidomics to track clonal dissemination and resistance–virulence convergence should be conducted[7,16,23-25]. Integrating molecular data with detailed clinical outcomes is essential to refine risk stratification, guide empirical treatment strategies, and improve patient prognosis[2,28,31].

This study demonstrates that *K. pneumoniae* strains circulating in the Çukurova region frequently exhibit both multidrug resistance and significant virulence, particularly within the K2 and K5 capsular serotypes. The strong association between carbapenemase/ESBL production and virulence gene carriage underscores the growing threat posed by carbapenem-resistant hypervirulent *K. pneumoniae* in hospital settings. These findings highlight the urgent need for routine molecular surveillance of capsular serotypes, virulence determinants, and resistance genes as integral components of infection-control programs, especially in intensive care units and other high-risk clinical services[33,34,36].

Author Contributions: Concept/Design : BB; Data acquisition: BB; Data analysis and interpretation: BB, HSB; Drafting manuscript: BB, HSB; Critical revision of manuscript: BB, HSB; Final approval and accountability: BB, HSB, FS; Technical or material support: BB; Supervision: FS; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained with the decision of the Ethics Committee of Hatay Mustafa Kemal University Non-

Interventional Clinical Research dated 27.08.2025 and numbered 11-45/9.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: For this study, the authors have no direct or indirect financial interest was declared.
Meeting Presentation: The authors have not presented this study as an oral presentation or poster at any congress or meeting. This study has not been published as a print or full article in any journal.

REFERENCES

1. Asokan S, Jacob T, Jacob J, AlSosowa AA, Cherian T, Peijnenburg WJGM et al. Klebsiella pneumoniae: A growing threat in the era of antimicrobial resistance. Microbe. 2025;7:100333.
2. Du C, Zhang H, Zhang Y, Zhang H, Zheng J, Liu C et al. Prognostic factors and nomogram for Klebsiella pneumoniae infections in intensive care unit. Infect Drug Resist. 2025;18:1237–51.
3. Itani R, Khojah HMJ, Kibrit R, Raychouni H, Shuhaiber P, Dib C et al. Risk factors associated with multidrug-resistant Klebsiella pneumoniae infections: A multicenter observational study in Lebanese hospitals. BMC Public Health. 2024;24:2958.
4. Moradi F, Akbari M, Vakili-Ghartavold R, Ostovari M, Hadi N. Molecular characterization of superbugs Klebsiella pneumoniae harboring extended-spectrum β-lactamase and carbapenemase resistance genes among hospitalized patients in southwestern Iran, Western Asia. Heliyon. 2024;10:e36858.
5. Ilyas R, Asghar S, Zehra M, Usmani Y, Khan RMA, Mirani ZA et al. Molecular assessment of carbapenem-resistant and ESBL Klebsiella pneumoniae clinical isolates to decipher the correlation of antimicrobial resistance with virulence traits. Infect Genet Evol. 2025;133:105785.
6. Soltani E, Hasani A, Ahangarzadeh Rezaee M, Zaare Nahandi M, Hasani A, Gholizadeh P. An alliance of carbapenem-resistant Klebsiella pneumoniae with precise capsular serotypes and clinical determinants: A disquietude in hospital setting. Can J Infect Dis Med Microbiol. 2022;2022:6086979.
7. Wang X, Wang J, Jiang X, Huang Z, Huang L, Wei Q et al. Molecular epidemiological analysis and research on resistance and virulence of carbapenem-resistant Klebsiella pneumoniae in a tertiary hospital from 2016 to 2023. BMC Microbiol. 2025;25:217.
8. Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT. A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. J Exp Med. 2004;199:697–705.
9. Singh RP, Kapoor A, Sinha A, Ma Y, Shankar M. Virulence factors of Klebsiella pneumoniae: Insights into canonical and emerging mechanisms driving pathogenicity and drug resistance. Microbe. 2025;7:100289.
10. Zierke L, Mourad R, Kohler TP, Müsken M, Hammerschmidt S. Influence of the polysaccharide capsule on virulence and fitness of Klebsiella pneumoniae. Front Microbiol. 2025;16:1450984.
11. Huang X, Li X, An H, Wang J, Ding M, Wang L et al. Capsule type defines the capability of Klebsiella pneumoniae in evading Kupffer cell capture in the liver. PLoS Pathog. 2022;18:e1010693.
12. Turton JF, Perry C, Elgohari S, Hampton CV. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat and virulence gene targets. J Med Microbiol. 2010;59:541–7.
13. Khrulnova S, Fedorova A, Frolova I, Tandilova K, Likold E, Klyasova G. Distribution of virulence genes and capsule types in Klebsiella pneumoniae among bloodstream isolates from patients with hematological malignancies. Diagn Microbiol Infect Dis. 2022;104:115744.
14. Yürek M, Cevahir N. Çeşitli klinik örneklerden izole edilen hipervirülan ve klasik Klebsiella pneumoniae izolatlarında virülans genleri ve karbapenem direnç genlerinin araştırılması. Mikrobiyol Bul. 2023;57:188–206.
15. Rastegar S, Moradi M, Kalantar-Neyestanaki D, Golabi Dehdasht A, Hosseini-Nave H. Virulence factors, capsular serotypes and antimicrobial resistance of hypervirulent Klebsiella pneumoniae and classical Klebsiella pneumoniae in Southeast Iran. Infect Chemother. 2023;53:e39.
16. Li X, Ma K, Liu X, Cheng P, Li L, Chai Y et al. Prevalence and molecular characterisation of multidrug-resistant ST11 hypervirulent Klebsiella pneumoniae in a teaching hospital. BMC Microbiol. 2025;25:352.
17. Abbas R, Chakkour M, Zein El Dine H, Obaseki EF, Obeid ST, Jezzini A et al. General overview of Klebsiella pneumoniae: Epidemiology and the role of siderophores in its pathogenicity. Biology (Basel). 2024;13:78.
18. Sahly H, Navon-Venezia S, Roesler L, Hay A, Carmeli Y, Podschun R et al. Extended-spectrum beta-lactamase production is associated with an increase in cell invasion and expression of fimbrial adhesins in Klebsiella pneumoniae. Antimicrob Agents Chemother. 2008;52:3029–34.
19. Turton JF, Baklan H, Siu LK, Kaufmann ME, Pitt TL. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in Klebsiella spp. and comparison of isolates within these serotypes. FEMS Microbiol Lett. 2008;284:247–52.
20. Araújo L, Papa-Ezdra R, Ávila P, Iribarnegaray V, Bado I, Telechea H et al. Great plasticity in a great pathogen: Capsular types, virulence factors and biofilm formation in ESBL-producing Klebsiella pneumoniae from pediatric infections in Uruguay. Antibiotics (Basel). 2024;13:170.
21. Yeh KM, Kurup A, Siu LK, Koh YL, Fung CP, Lin JC et al. Capsular serotype K1 or K2, rather than magA and rmpA, is a major virulence determinant for

Klebsiella pneumoniae liver abscess in Singapore and Taiwan. J Clin Microbiol. 2007;45:466–71.
22. Huo B, Liu Y, Fan L, Xu L, Yang W, Wei D et al. Mechanistic insights into the formation and spread of carbapenem-resistant hypervirulent Klebsiella pneumoniae via an IncHI1B/repB hybrid virulence plasmid. Microb Genom. 2025;11:001534.
23. Di Pilato V, Pollini S, Miriagou V, Rossolini GM, D'Andrea MM. Carbapenem-resistant Klebsiella pneumoniae: The role of plasmids in emergence, dissemination, and evolution of a major clinical challenge. Expert Rev Anti Infect Ther. 2024;22:25–43.
24. Shapovalova VV, Chulkova PS, Ageevets VA, Nurmukanova V, Verentsova IV, Girina AA et al. High-risk lineages of hybrid plasmids carrying virulence and carbapenemase genes. Antibiotics (Basel). 2024;13:1224.
25. Yang X, Dong N, Liu X, Yang C, Ye L, Chan EWC et al. Co-conjugation of virulence plasmid and KPC plasmid in a clinical Klebsiella pneumoniae strain. Front Microbiol. 2021;12:739461.
26. Bulman ZP, Tan X, Chu TY, Huang Y, Rana AP, Singh N et al. Ceftazidime-avibactam–based combinations against carbapenemase-producing Klebsiella pneumoniae harboring hypervirulence plasmids. Comput Struct Biotechnol J. 2022;20:3946–54.
27. Hleba L, Hlebová M, Kováčik A, Čuboň J, Medo J. Carbapenemase-producing Klebsiella pneumoniae (KPC): What is the best MALDI-TOF MS detection method? Antibiotics (Basel). 2021;10:1549.
28. Moraes LS, Magalhaes GLG, Soncini JGM, Pelisson M, Perugini MRE, Vespero EC. High mortality from carbapenem-resistant Klebsiella pneumoniae bloodstream infection. Microb Pathog. 2022;167:105519.
29. Tavassoli N, Zaboli F, Khoshbakht R, Khosravi M. Epidemiological and clinical insights into genotypic diversity, antibiotic resistance determinants, and capsular types of Klebsiella pneumoniae isolates from hospitalized patients in Amol, Northern Iran. Avicenna J Clin Microbiol Infect. 2025;12:10–20.
30. Baykara B, Cimentepe M, Kandemir T, Koksal F. Investigation of the relationship between colistin resistance and capsule serotypes in carbapenem-resistant Klebsiella pneumoniae strains. New Microbiol. 2022;45:124–9.
31. Enciu O, Toma EA, Calu V, Pîrîianu DC, Poroşnicu AL, Miron A et al. Klebsiella pneumoniae invasive liver abscess syndrome: Experience of a single center and up-to-date review of the literature. Diagnostics (Basel). 2025;15:1533.
32. Liu Y, Liu C, Zheng W, Zhang X, Yu J, Gao Q et al. PCR detection of Klebsiella pneumoniae in infant formula based on 16S–23S internal transcribed spacer. Int J Food Microbiol. 2008;125:230–5.
33. El Fertas-Aissani R, Messai Y, Alouache S, Bakour R. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathol Biol (Paris). 2013;61:209–16.
34. Dorman MJ, Feltwell T, Goulding DA, Parkhill J, Short FL. The capsule regulatory network of Klebsiella pneumoniae defined by density-TraDISort. mBio. 2018;9:e01863-18.
35. Martin RM, Bachman MA. Colonization, infection, and the accessory genome of Klebsiella pneumoniae. Front Cell Infect Microbiol. 2018;8:4.
36. Pariseau DA, Ring BE, Khadka S, Mike LA. Cultivation and genomic DNA extraction of Klebsiella pneumoniae. Curr Protoc. 2024;4:e932.
37. Mamlouk K, Boutiba-Ben Boubaker I, Gautier V, Vimont S, Picard B, Ben Redjeb S et al. Emergence and outbreaks of CTX-M β-lactamase-producing Escherichia coli and Klebsiella pneumoniae strains in a Tunisian hospital. J Clin Microbiol. 2006;44:4049–56.