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Virulence genes, efflux pumps, and molecular typing of *Klebsiella pneumoniae* isolates from North Iran

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Abstract

Resistant and virulent strains of *Klebsiella pneumoniae* (*K. pneumoniae*) are rapidly disseminated among both hospitalized patients and communities, therefore, the identification of the genes responsible for virulence and resistance, along with the clonal relatedness of these strains, could be beneficial in the management of the dissemination of these strains among patients. The aim of the present study was to assess antibiotic susceptibility, virulence and resistance genes, as well as the genetic relatedness of *K. pneumoniae* strains isolated from patients admitted to four hospitals in Mazandaran, Iran. A total of 95 *K. pneumoniae* were gathered from hospitalized patients. All isolates were confirmed using biochemical and conventional microbiological methods, followed by the assessment of susceptibility patterns through disk diffusion and the detection of resistance and virulence genes using conventional PCR. The genetic diversity of clinical isolates was determined using the Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) technique. The resistance frequencies varied, with the highest being for ampicillin/sulbactam (95.8%) and the lowest for fosfomycin (3.2%). Only one strain displayed a non-MDR profile against all antibiotics tested. Virulence-associated genes were detected, such as *mrkD* (90.5%), *fimH* (80%), *entB* (92.6%), *iutA* (25.3%), and *ybtS* (68.4%). Genes associated with efflux pumps and outer membrane porins included *acrAB* (98.9%), *tolC* (95.8%), *mdtK* (83.2%), *ompK35* (95.8%), and *ompK36* (92.6%). Based on ERIC-PCR patterns with a 90% similarity, the isolates were categorized into 17 distinct clusters. While the majority of isolates had a same profile and were grouped in the predominant pattern, 11 isolates were identified as singletons. Our study indicates that the prevalence of MDR *K. pneumoniae* carrying virulence genes and exhibiting close relatedness underscores the urgent need for effective strategies to control and prevent infections caused by *K. pneumoniae*.

Keywords *Klebsiella pneumoniae*, Multidrug Resistance, Virulence factor, Efflux pumps, Outer membrane porins

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative opportunistic pathogen associated with a wide range of infections, from mild urinary tract infections (UTIs) to severe bacteremia and pneumonia, which can lead to serious complications and even death, especially in hospitalized or otherwise immunocompromised individuals (Jian-Li et al. 2017; Ferreira et al. 2019; Türkel et al. 2018; Compain et al. 2014). In addition, a recent systematic review and meta-analysis revealed that *K. pneumoniae* is a significant cause of neonatal sepsis in Iran, with a pooled prevalence estimate of 23.2% among gram-negative bacteria (Moftian et al. 2023). The overuse use of antibiotics plays an important role in the emergence of multidrug-resistant (MDR) and even extensively drug-resistant (XDR) bacteria, and the treatment of these infections has become a major problem in recent years due to their resistance to broad-spectrum antibiotics (Lerminiaux and Cameron 2019; Doorduijn et al. 2016; Struve and Krogfelt 2003). *K. pneumoniae* has been designated by the World Health Organization (WHO) as a species requiring prioritized research on new antibiotics (Shrivastava et al. 2018). The pathogenesis of *K. pneumoniae* is closely associated with a diverse array of virulence genes that significantly enhance its capacity to infect and persist within the host environment (Calhau et al. 2014; Wasfi et al. 2016). The fimbrial genes *mrkD* (type 3 fimbrial adhesin) and *fimH-1* (type 1 fimbrial adhesin) independently contribute to pathogenicity by facilitating bacterial adherence to host cells (Riwu et al. 2022). Furthermore, genes such as *entB* (enterobactin biosynthetic gene), *iutA* (aerobactin receptor gene), and *ybtS* (yersiniabactin biosynthetic gene) are involved in iron metabolism and significantly boosted the virulence potential of the *K. pneumoniae* strains (Tapia-Pastrana et al. 2024). Notably, most strains of the hypermucoviscous phenotype related to the K1 and K2 capsular serotypes, and the main fundamental mechanisms are due to the existence of mucoviscosity-associated gene A (*magA*) and *wcaG* (encoding GDP-fucose synthetase) and regulator of mucoid phenotype A (*rmpA*) genes. Frequency studies indicated a statistical correlation of *rmpA* with *K. pneumoniae* virulence (Imai et al. 2019; Lin et al. 2020).

In addition tovirulence factors, the presence of genes associated with efflux pumps and outer membrane porins is vital for conferring antibiotic resistance. Genes coding for the multicomponent drug pumps AcrAB-TolC and *mdtK* and porin coding genes (*ompK35* and *ompK36*) have high clinical importance in the resistance to multiple antimicrobial agents, enabling bacteria to survive, particularly during the infections caused by *K. pneumoniae* (Ranjbar et al. 2019; Chetri et al. 2019).

Several studies have shown that antibiotic-resistant *K. pneumoniae* strains are a major cause of infections acquired in healthcare settings and communities, mainly due to the increase in high-risk clonal lineages (Heiden et al. 2020; Qiu et al. 2021). The global concern lies in the spread of virulent and MDR clones of *K. pneumoniae*, highlighting the importance of identifying and controlling these strains in hospitals, as well as managing their transmission in communities and identifying sources of infection (Wyres et al. 2020).

Due to the scarcity of data on the genetic relatedness of *K. pneumoniae* isolates and their correlation with resistance and virulence factors in nosocomial infections in Iran, the primary aim of this study was to evaluate the pathogenicity and antibiotic resistance profiles to determine the MDR and XDR phenotypes, as well as to investigate the genetic diversity among *K. pneumoniae* strains isolated from hospitalized individuals in the teaching hospitals located in northern region of Iran.

Methods

Sampling and bacterial identification

This study was conducted on 95 *K. pneumoniae* obtained from clinical samples of patients admitted to four hospitals affiliated with Mazandaran University of Medical Sciences (Imam Khomeini, Bu-Ali Sina, Razi, and Zare hospitals) in Iran from August 2018 to August 2019. Specimens were collected from various clinical sites, including blood, sputum, urine, wound, and tissue. First, bacteria were cultured on blood agar and Macconkey agar media (Merck, Germany). Then, all *K. pneumoniae* isolates were confirmed using traditional biochemical tests, including oxidase, catalase, indole, citrate, methyl red, Voges-Proskauer, urease, and Kligler iron agar (KIA) tests (Hall 1995; Podschun and Ullmann 1998) and the API 20E kit (bioMérieux, La-Balme-les-Grottes, France). The purified isolates were stored in trypticase soy broth (TSB) (Merck, Germany) with 20% glycerol at -80°C for future use. Furthermore, the demographic and clinical information of the patients were collected from their medical records. The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (Ethical ID: IR.MAZUMC.REC.1399.658), and all the applied methods were in accordance with the Declaration of Helsinki.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was conducted with the disk diffusion method in accordance with the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI 2021) utilizing a panel of disks containing various antibiotics (MAST Diagnostics, UK): levofloxacin (LEV; 5 µg), ceftazidime (CAZ; 30 µg), cefotaxime (CTX; 30 µg), cefepime (FEP; 30 µg), amikacin (AK; 30 µg), gentamicin (GM; 10 µg), imipenem (IPM; 10 µg), ertapenem (ETP; 10 µg), meropenem (MER; 10 µg), ceftriaxone

(CRO; 30 µg), ampicillin/sulbactam (SAM; 10/10 µg), cefoperazone (CFP; 75 µg), nitrofurantoin (NIT;300 µg), ciprofloxacin (CIP; 5 µg), tetracycline (TET; 30 µg), and trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), fosfomycin (FOS; 200 µg), cefazolin (CZ; 30 µg), aztreonam (ATM; 30 µg), tobramycin (TOB; 10 µg), ceftaroline (CPT; 30 µg), piperacillin-tazobactam (TPZ; 100/10 µg), (MAST Diagnostics, Merseyside, UK). In this study, MDR strains were recognized as non-susceptible to ≥1 agent in ≥3 antimicrobial categories, however XDR strains are known to be non-susceptible to ≥1 agent in all but ≤2 categories (Magiorakos et al. 2012),

Molecular methods

Genomic DNA extraction from the isolates was extracted according to the manufacturer's protocol of the DNA extraction kit (SinaPureTM, SinaClon, Iran). The amplification of virulence and resistance genes (*rmpA*, *fimH*, *mrkD*, *iutA*, *entB*, *ybtS*, *K1*, *K2*, *tolC*, *acrAB*, *mdtK*, *ompK35,* and *ompK36*) was conducted through polymerase chain reaction (PCR), with a no-template control included to detect any contamination in the PCR reagents (as a negative control). The reaction mixture for PCR amplification was prepared in a total volume of 25 µL, containing 12.5 µL of 2X Master Mix (Ampliqon, Denmark), 0.5 µL of forward primer (10 pmol), 0.5 µL of reverse primer (10 pmol), 3 µL of template DNA (30–50 ng), and 8.5 µL of RNase/DNase free water. Subsequently, the amplicons were subjected to electrophoresis in a 1% agarose gel and visualization was achieved after staining with a safe stain. A 100 bp ladder (Fermentase, Vilnius, Lithuania) served as the molecular weight marker. The primer sequences are demonstrated in Table 1.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR fingerprinting

Genomic DNA of the isolates was also used as a template to amplify sequences for ERIC PCR. PCR was performed in a final volume of 25 µL including contained 12.5 µL PCR Master Mix (Ampliqon, Denmark), 0.5 µL of each primer (forward and reverse), and 3 µL of template DNA (30–50 ng). The reaction mixture was initially denatured at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 52°C for 1 min, and extension at 70 °C for 10 min, with final extension at 72 °C for 20 min. *P. aeruginosa* ATCC 27,853 was used as a positive control.

Clonality of the strains was determined by ERIC-PCR fingerprinting and the obtained dendrogram was analyzed with Gelcompar II software, version 5.1 (Applied Maths, Belgium). Similarities in amplicon profiles were compared using a Dice coefficient at 1.2% tolerance and

Table 1 The list of primers sequences used in the study

Gene	Primer sequence (5´–3´), F/*R*	Amplicon size (bp)	Annealing temperature (°C)	Reference
rmpA	ACTGGGCTACCTCTGCTTCA	535	54	Ballén et al. (2021)
	CTTGCATGAGCCATCTTTCA			
iutA	GGGAAAGGCTTCTCTGCCAT	920	60	Chetri et al. (2019)
	TTATTCGCCACCACGCTCTT			
entB	GTCAACTGGGCCTTTGAGCCGTC	400	68	Chetri et al. (2019)
	TATGGGCGTAAACGCCGGTGAT			
ybtS	GACGGAAACAGCACGGTAAA	242	58	Chetri et al. (2019)
	GAGCATAATAAGGCGAAAGA			
K1	GGTGCTCTTTACATCATTGC	1283	59	Hall (1995)
	GCAATGGCCATTTGCGTTAG			
K2	GGATTATGACAGCCTCTCCT	908	58	Hall (1995)
	CGACTTGGTCCCAACAGTTT			
TolC	ATCAGCAACCCCGATCTGCGT	525	65	Hall (1995)
	CCGGTGACTTGACGCAGTCCT			
acrAB	ATCAGCGGCCGGATTGGTAAA	312	65	Hall (1995)
	CGGGTTCGGGAAAATAGCGCG			
mdtK	GCGCTTAACTTCAGCTCA	435	49	Hall (1995)
	GATGATAAATCCACACCAGAA			
ompK35	CTCCAGCTCTAACCGTAGCG	241	61	Hall (1995)
	GGTCTGTACGTAGCCGATGG			
ompK36	GAAATTTATAACAAAGACGGC	305	57	Hall (1995)
	GACGTTACGTCGTATACTACG			
fimH	TGCTGCTGGGCTGGTCGATG	550	65	Ballén et al. (2021)
	GGGAGGGTGACGGTGACATC			
mrkD	CCACCAACTATTCCCTCGAA	226	58	Hall (1995)
	ATGGAACCCACATCGACATT			

1% optimization, and a dendrogram was constructed using the unweighted-pair group (UPGMA) method, with a cut off of 90% similarity.

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). The ANOVA/Chi-square test utilized to establish and verify the presence of a significant distinction between the frequency of the target genes and resistance to specific antibiotics. A p-value less than 0.05 was considered as statistically significant.

Results

Study population

A total of 95 patients, including 42 (44.2%) males and 53 (55.8%) females, with an average age of 50.91 ± 18.4 (5–85) years, underwent examination. Among them, 38 were admitted at Imam Khomeini Hospital (40%), 44 at Bu Ali Sina Hospital (46.3%), 6 at Zare Hospital (6.3%), and 7 at Razi Hospital (7.4%). Regarding sample distribution, 60 samples were urine (63.16%), 9 were blood (9.47%), 15 were tissue samples (15.79%), 7 were wound drainage (7.37%), and 4 were sputum (4.21%).

Antimicrobial resistance pattern

Our study revealed varying rates of resistance to different antibiotics among *K. pneumoniae* isolates: ampicillin/sulbactam (95.8%), cefazolin (85.3%), ceftaroline (73.7%), aztreonam (69.5%), piperacillin-tazobactam (63.2%), nitrofurantoin (62.1%), tobramycin (60%), cefoperazone (55.8%), cefotaxime (52.6%), trimethoprim-sulfamethoxazole (52.6%), ceftazidime (47.4%), ceftriaxone (46.3%), cefepime (46.3%), meropenem (44.2%), ciprofloxacin (35.8%), imipenem (33.7%), levofloxacin (31.6%), tetracycline (28.4%), gentamicin (24.2%), ertapenem (24.2%), amikacin (9.5%), and fosfomycin (3.2%). The highest and lowest resistance frequencies were observed for ampicillin/sulbactam and fosfomycin, respectively. A significant majority of the isolates were identified as MDR (93.7%), while XDR and non-MDR isolates accounted for 5.3% and 1.1% of the isolates, respectively.

Virulence-profiling

Table 2 illustrates the prevalence of resistance genes in various clinical specimens. Virulence-associated genes were detected, such as *mrkD* (90.5%), *fimH* (80%), *entB* (92.6%), *iutA* (25.3%), and *ybtS* (68.4%). Genes associated with efflux pumps and outer membrane porins included *acrAB* (98.9%), *tolC* (95.8%), *mdtK* (83.2%), *OmpK35* (95.8%), and *OmpK36* (92.6%). Among the isolates, a total of 13 genes were identified, with *acrAB* showing the highest rate at 98.9%, followed by *tolC*. Nonetheless, neither *rmpA* nor *K1* were detected in any of the isolates. Isolates from urine displayed a higher number of resistant genes in this study. Table 3 displays the distribution of resistance genes according to the patients' age. The data in this table reveals that patients aged between 45 and 60 years exhibited the highest rate of resistant genes, whereas children showed the lowest rate.

Our findings revealed that 8.42% of the isolates had all resistance genes altogether, with the highest rate attributed to the co-occurrence of 9 genes among the isolates (Fig. 1).

Molecular typing

According to the dendrogram obtained in the present study based on a cut off similarity value of 90%, the isolates were classified into 17 distinct clusters. The clonal relatedness, source of isolates, and antimicrobial resistance patterns are summarized in Fig. 2. Notably, the majority of isolates (48 strains) shared the same profile and were grouped into a predominant pattern, 11 isolates appeared to be singletons and had a unique fingerprint

Table 2 Distribution of the virulence, antibiotic resistance and serotypes genes in *K. pneumoniae* isolated from clinical samples

Genes	Specimens (%)					
	Blood	Sputum	Tissue	Urine	Wound	Total
ompK36	9(10.2)	3(3.4)	12(13.6)	58(65.9)	6(6.8)	88(92.6)
tolC	8(8.8)	4(4.4)	14(15.4)	58(63.7)	7(7.7)	91(95.8)
acrAB	9(9.6)	4(4.3)	15(16.0)	59(62.8)	7(7.4)	94(98.9)
ompK35	9(9.9)	4(4.4)	13(14.3)	58(63.7)	7(7.7)	91(95.8)
iutA	4(16.7)	1(4.2)	2(8.3)	15(62.5)	2(8.3)	24(25.3)
ybtS	2(3.1)	3(4.6)	10(15.4)	43(66.2)	7(10.8)	65(68.4)
entB	7(8.0)	4(4.5)	13(4.8)	57(64.8)	7(8.0)	88(92.6)
fimH	6(7.9)	4(5.3)	12(15.8)	48(63.2)	6(7.9)	76(80.0)
mdtK	7(8.9)	3(3.8)	13(16.5)	49(62.0)	7(8.9)	79(83.2)
K2	6(9.4)	2(3.1)	11(17.2)	39(60.9)	6(9.4)	64(67.4)
K1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
rmpA	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
mrkD	8(9.3)	4(4.7)	12(14.0)	55(64.0)	7(8.1)	86(90.5)

Table 3 Association between the age of patients and the genes detected in this research

Genes	Age, N (%)					
	>15	15–30	30–45	45–60	60–75	≥75
OmpK36						
	3(3.4)	8(9.1)	18(20.5)	32(36.4)	16(18.2)	11(12.5)
tolC						
	3(2.3)	9(9.9)	21(23.1)	31(34.1)	16(17.6)	11(12.1)
acrAB						
	3(3.2)	9(9.6)	21(22.3)	34(36.2)	15(16.0)	12(12.8)
ompK35						
	3(3.2)	8(8.8)	20(22.0)	33(36.3)	16(17.6)	11(12.1)
iutA						
	1(4.2)	2(8.3)	6(25.0)	10(41.7)	2(8.3)	3(12.5)
ybtS						
	1(1.5)	6(9.2)	13(20.0)	27(41.5)	10(15.4)	8(12.3)
entB						
	2(2.3)	9(10.2)	20(22.7)	31(35.2)	15(17.0)	11(12.5)
fimH						
	1(1.3)	8(10.5)	16(21.1)	34(35.8)	16(16.8)	12(12.6)
mdtK						
	1(1.3)	7(8.9)	17(21.5)	29(36.7)	14(17.7)	11(13.9)
K2						
	2(4.7)	6(9.4)	16(25.0)	20(31.3)	10(15.6)	9(14.1)
K1						
	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
rmpA						
	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
mrkD						
	2(2.3)	9(10.5)	20(23.3)	30(34.9)	15(17.4)	10(11.6)

that were unrelated to the other isolates. The vast majority of the isolates ($n=48$, approximately 50%) belonged to the predominant pattern that showed only one band in ERIC-PCR. Additionally, 17 strains were clustered together in a smaller group. Three clusters consisted of four isolates each, while one cluster encompassed three isolates. Also, there was a distinct group comprising only two similar isolates.

Discussion

Antibiotic resistance emerges when bacteria alter in response to medication use, posing a significant threat to public health globally. This trend leads to prolonged hospitalizations and rising mortality rates, affecting various societies. The prevalence of antibiotic resistance is escalating perilously worldwide, as indicated by numerous studies highlighting the high rates of resistance among *K. pneumoniae* isolates in Iran (Nasehi et al. 2010; Shoeib et al. 2011; Vaez et al. 2019). In our study, the highest and lowest resistance frequencies were observed for ampicillin/sulbactam and fosfomycin, respectively. A considerable majority of the isolates were classified as MDR (93.7%), while XDR and non-MDR isolates constituted 5.3% and 1.1% of the samples. Similarly, Saki et al. (2019) demonstrated a high MDR percentage among *K. pneumoniae* isolates. Moreover, in a study conducted in Spain in 2021, 40.16% of the samples were MDR and 1.57% were XDR, and the resistance in the samples isolated from urine to ciprofloxacin was 42.11%, amoxicillin clavulanic acid 36.48%, trimethoprim-sulfamethoxazole 36.48%, ceftazidime 33.33%, and fosfomycin 24.56% (Ballén et al. 2021).

The high frequency of MDR and XDR *K. pneumoniae* observed in this study underscores the urgent need for enhanced surveillance and infection control strategies in healthcare settings. Routine molecular surveillance and antimicrobial resistance monitoring are critical for tracking the spread of resistant strains. Additionally, the Antimicrobial Stewardship Programs (ASPs) should be reinforced to optimize antimicrobials use and minimize the selection pressure driving resistance. Strict infection prevention measures, including hand hygiene protocols, environmental disinfection, and patient isolation strategies, are essential to limit the transmission of MDR/XDR strains in hospitals (Lee et al. 2023).

In the study by Derakhshan et al., the highest and lowest percentages of resistance in *K. pneumoniae* were related to amoxicillin clavulanate (60.5%) and cefoxitin (7.5%), respectively (Derakhshan et al. 2016). Sanikhani and colleagues also reported significant resistance rates,

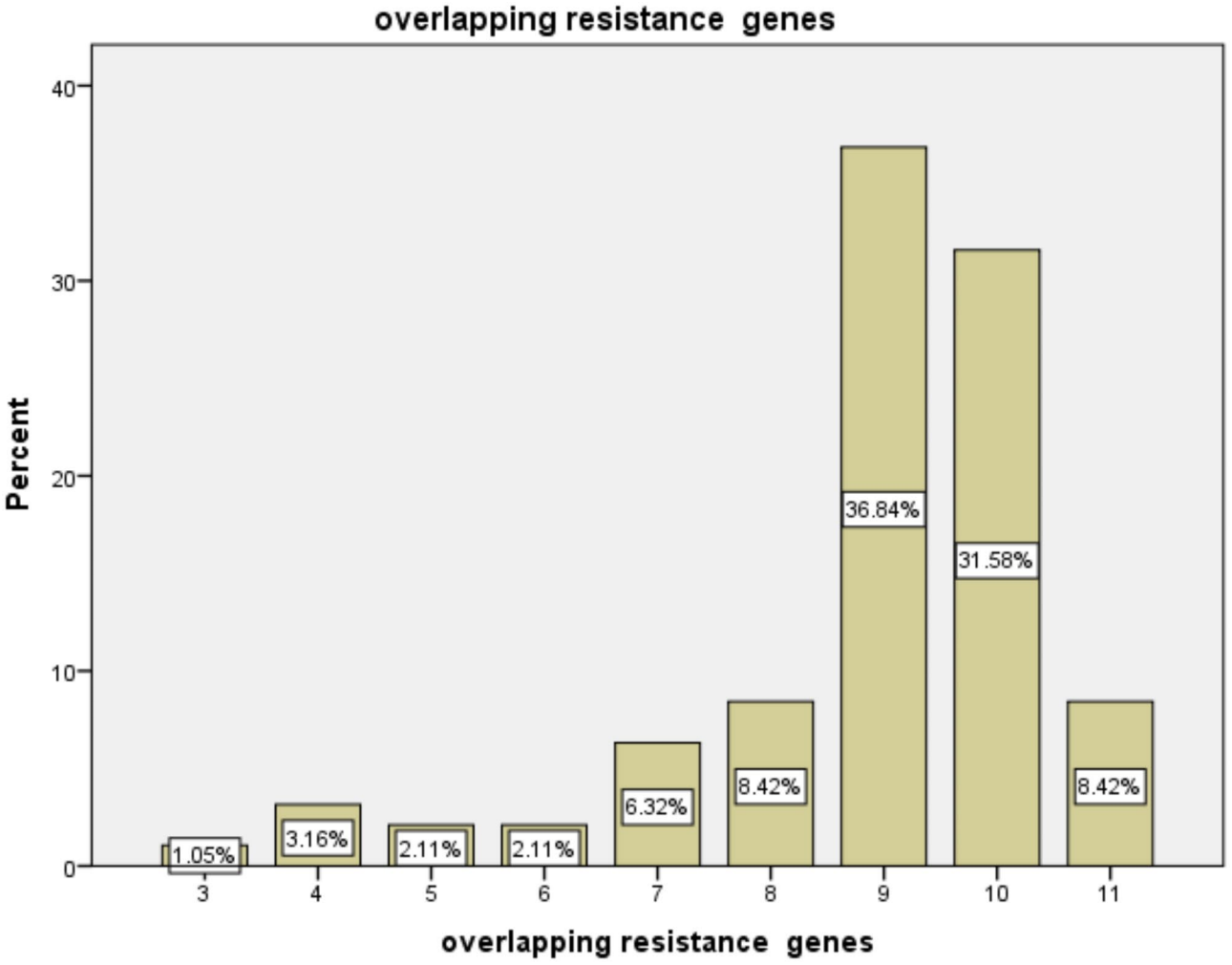
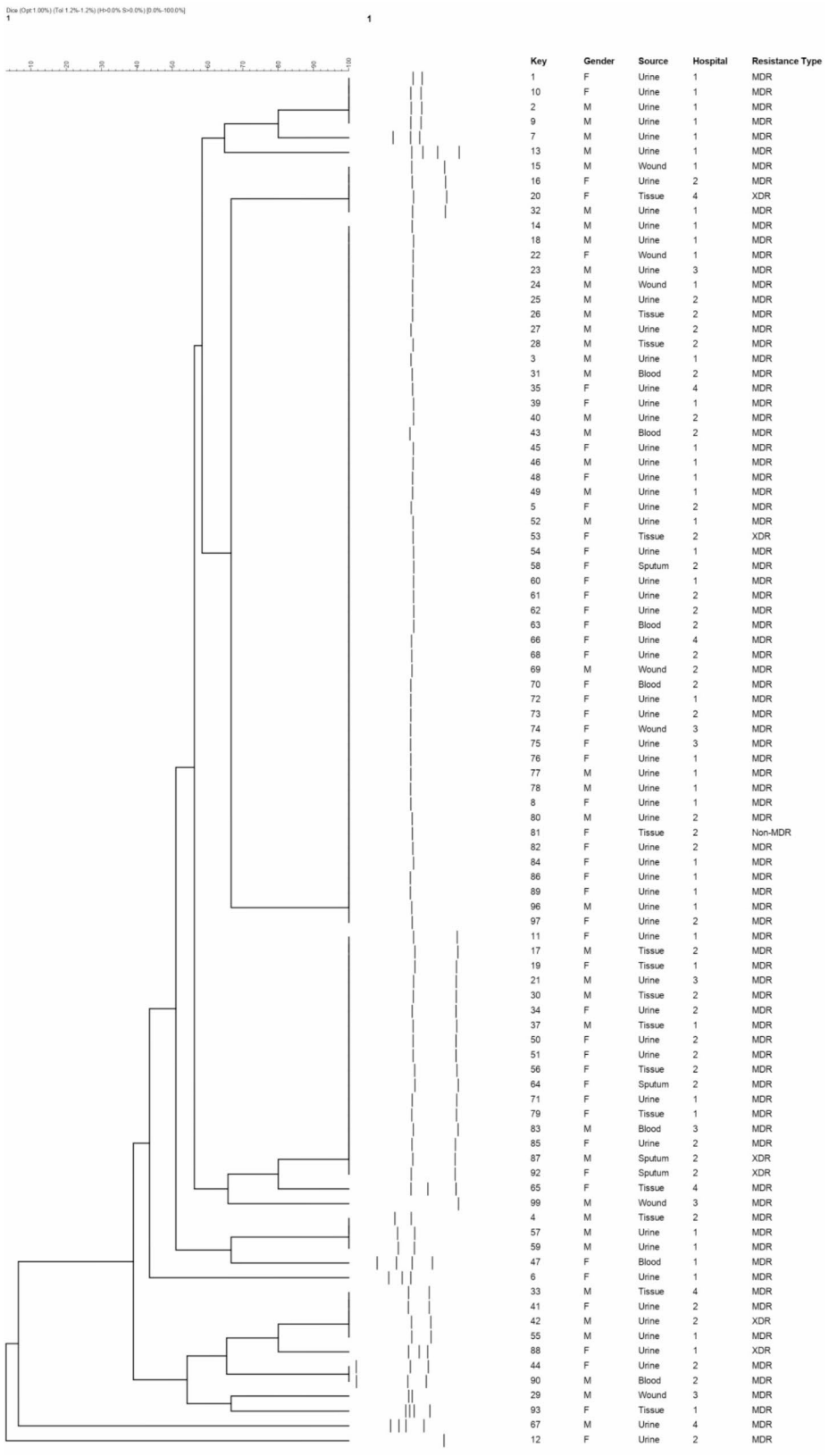


Fig. 1 The frequency of several virulence genes co-occurrence among *K. pneumoniea* isolates

with ertapenem and fosfomycin displaying maximum and minimum resistance frequencies, respectively (Sanikhani et al. 2021). According to Bina et al.'s findings, a notable proportion of *K. pneumoniae* strains exhibited resistance to piperacillin and carbapenems (Bina et al. 2015). A study from Pakistan in 2020 found higher resistance rates to ciprofloxacin (53%), gentamicin (55%) and fosfomycin (22%) compared to our results (Imtiaz et al. 2021).

Global data also highlights variations in antibiotic resistance among *K. pneumoniae* isolates, largely due to differences in antibiotic usage guidelines across countries, such as higher fosfomycin resistance rate in Portugal (93.5%) (Machado et al. 2006), in compared to our study.

A study conducted at Rize State Hospital in Turkey reported high resistance rates to ampicillin (100%), ertapenem (100%), and amoxicillin-clavulanic acid (98.4%), while resistance to amikacin (1.7%) and colistin (0%) was notably low (Karaman et al. 2024). In contrast, our study found the highest resistance to ampicillin/sulbactam (95.8%) and cefazolin (85.3%), while fosfomycin (3.2%) and amikacin (9.5%) exhibited the lowest resistance rates. Carbapenem resistance in our isolates (meropenem 44.2%, imipenem 33.7%, ertapenem 24.2%) was lower than that reported in the Karaman study (meropenem 96.7%, imipenem 85.04%, ertapenem 76.7%). These differences highlight regional variations in resistance patterns, likely influenced by local antibiotic prescribing practices and infection control strategies. This research revealed that the *acrAB* gene had the highest frequency among the investigated factors. Notably, none of the isolates harbored the *rmpA* gene, which regulates the mucoid phenotype in *K. pneumoniae*. Furthermore, *K. pneumoniae* isolates in this study displayed the K2 serotype, while the K1 serotype was not detected, consistent with previous studies reporting a low frequency of the K1 capsule type (Ferreira et al. 2019; Sedighi et al. 2020). The findings of this study suggest that the virulence genes play a crucial role in the pathogenesis of *K. pneumoniae* strains, as the results of the molecular



Key	Gender	Source	Hospital	Resistance Type
1	F	Urine	1	MDR
10	F	Urine	1	MDR
2	M	Urine	1	MDR
9	M	Urine	1	MDR
7	M	Urine	1	MDR
13	M	Urine	1	MDR
15	M	Wound	1	MDR
16	F	Urine	2	MDR
20	F	Tissue	4	XDR
32	M	Urine	1	MDR
14	M	Urine	1	MDR
18	M	Urine	1	MDR
22	F	Wound	1	MDR
23	M	Urine	3	MDR
24	M	Wound	1	MDR
25	M	Urine	2	MDR
26	M	Tissue	2	MDR
27	M	Urine	2	MDR
28	M	Tissue	2	MDR
3	M	Urine	1	MDR
31	M	Blood	2	MDR
35	F	Urine	4	MDR
39	F	Urine	1	MDR
40	M	Urine	2	MDR
43	M	Blood	2	MDR
45	F	Urine	1	MDR
46	M	Urine	1	MDR
48	F	Urine	1	MDR
49	M	Urine	1	MDR
5	F	Urine	2	MDR
52	M	Urine	1	MDR
53	F	Tissue	2	XDR
54	F	Urine	1	MDR
58	F	Sputum	2	MDR
60	F	Urine	1	MDR
61	F	Urine	2	MDR
62	F	Urine	2	MDR
63	F	Blood	2	MDR
66	F	Urine	4	MDR
68	F	Urine	2	MDR
69	M	Wound	2	MDR
70	F	Blood	2	MDR
72	F	Urine	1	MDR
73	F	Urine	2	MDR
74	F	Wound	3	MDR
75	F	Urine	3	MDR
76	F	Urine	1	MDR
77	M	Urine	1	MDR
78	M	Urine	1	MDR
8	F	Urine	1	MDR
80	M	Urine	2	MDR
81	F	Tissue	2	Non-MDR
82	F	Urine	2	MDR
84	F	Urine	1	MDR
86	F	Urine	1	MDR
89	F	Urine	1	MDR
96	M	Urine	1	MDR
97	F	Urine	2	MDR
11	F	Urine	1	MDR
17	M	Tissue	2	MDR
19	F	Tissue	1	MDR
21	M	Urine	3	MDR
30	M	Tissue	2	MDR
34	F	Urine	2	MDR
37	M	Tissue	1	MDR
50	F	Urine	2	MDR
51	F	Urine	2	MDR
56	F	Tissue	2	MDR
64	F	Sputum	2	MDR
71	F	Urine	1	MDR
79	F	Tissue	1	MDR
83	M	Blood	3	MDR
85	F	Urine	2	MDR
87	M	Sputum	2	XDR
92	F	Sputum	2	XDR
65	F	Tissue	4	MDR
99	M	Wound	3	MDR
4	M	Tissue	2	MDR
57	M	Urine	1	MDR
59	M	Urine	1	MDR
47	F	Blood	1	MDR
6	F	Urine	1	MDR
33	M	Tissue	4	MDR
41	F	Urine	2	MDR
42	M	Urine	2	XDR
55	M	Urine	1	MDR
88	F	Urine	1	XDR
44	F	Urine	2	MDR
90	M	Blood	2	MDR
29	M	Wound	3	MDR
93	F	Tissue	1	MDR
67	M	Urine	4	MDR
12	F	Urine	2	MDR

Fig. 2 Dendrogram representing the genetic relationship among *K. pneumoniae* strains isolated from different clinical samples of patients admitted to four hospitals

analysis were largely similar to those of previous studies (Mirzaie and Ranjbar 2021). Nonetheless, variations in gene frequencies among different studies may reflect differences in sample origin, geographical location, and the number of isolates included in the analysis.

Interestingly, a higher prevalence of virulence and resistance genes was observed in urine samples compared to other clinical specimens, highlighting the significant role of these genetic factors in the development of UTI. Similarly, research by Ballen et al. showed that *K. pneumoniae* strains from urine cultures had increased antimicrobial resistance, produced ESBLs, and formed biofilms more than isolates from respiratory or blood samples (Ballén et al. 2021). A comprehensive assessment of antimicrobial resistance patterns, biofilm formation, and virulence factors in *K. pneumoniae* is essential for understanding the pathogenic mechanisms of this bacterium in clinical settings and for improving treatment strategies. Thus, implementing active surveillance programs that target both antimicrobial resistance and virulence factors is crucial to prevent the spread and growth of highly virulent or extensively resistant strains.

While various typing methods such as ribotyping, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing (MLST) are commonly used for bacterial genotyping, they are often costly and time-consuming. In contrast, ERIC-PCR, provide a quick, reliable, and cost-effective alternative for typing among the Enterobacterales. ERIC-PCR represents a practical and accessible option, enabling the discrimination of bacterial strains without the complexity and expense associated with other traditional methods (Sedighi et al. 2020). Moreover, studies have shown that ERIC-PCR offers a discrimination power comparable to that of PFGE (Kundu et al. 2022).

Based on the ERIC-PCR results, strains grouped in a cluster may not all come from the same hospital or source. Only cluster number 1 showed strains with the same origin and source. This suggests that MDR and XDR resistant strains are disseminating in hospitals, where strains with similar patterns were found in different hospitals and patients. Surprisingly, nearly half of the *K. pneumoniae* strains had the same profile which is grouped in predominant pattern, despite originating from different sources and even one isolate being non-MDR and susceptible to antibiotics. Consequently, strains with 100% genetic similarity could be found in different hospitals and patients.

Surprisingly, nearly half of the *K. pneumoniae* strains had the same profile which is grouped in predominant pattern, despite originating from different sources and even one isolate being non-MDR and susceptible to antibiotics. Consequently, strains with 100% genetic similarity could be found in different hospitals and patients.

In a study in Brazil, the genetic resemblance among 25 *K. pneumoniae* isolates from the ICU was examined using the ERIC-PCR test, revealing two clusters with over 70% genetic similarity. Additionally, three isolates shared 100% genetic similarity, while four isolates did not include in any cluster (Ferreira et al. 2019). In another study by Sedighi and colleagues, the genetic similarity of 86 *K. pneumoniae* isolates was explored, resulting in 39 ERIC patterns, with 17 patterns being similar among different isolates and 22 patterns being unique. This study's findings highlighted significant genetic variation among *K. pneumoniae* strains, indicating polyclonal dissemination among this bacterium's isolates across hospitals (Sedighi et al. 2020).

In this study, the high prevalence of *K. pneumoniae* strains resistant to most antibiotics was found, with over 90% of isolates being MDR. The *acrAB* had the highest rate among studied genes, while neither *rmpA* nor *K1* were found in any of the isolates. Furthermore, urine samples showed a higher prevalence of virulence and resistance genes compared to other clinical specimens. Additionally, almost half of the *K. pneumoniae* strains had the same profile, despite originating from different sources, with one isolate being non-MDR and susceptible to antibiotics. However, strains with 100% genetic similarity were identified in various hospitals and patients. Considering the crucial role of *K. pneumoniae* in nosocomial infections, it is clear that findings from these studies can significantly contribute to reducing hospitalizations, treatment costs, and patient mortality. This study has some limitations. Sequencing was not performed to confirm resistance and virulence genes due to budget constraints. Additionally, the sample size was limited to 95 isolates from a single region, which may affect generalizability.

Therefore, future research should focus on exploring the molecular mechanisms of resistance and virulence in *K. pneumoniae*, using advanced sequencing techniques, investigating novel resistance mechanisms, and understanding the link between virulence genes and clinical outcomes. Additionally, new therapeutic strategies, environmental transmission, and global epidemiological studies should be further explored to combat resistant strains.

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Authors' contribution

AHB: Performed the experiments, wrote the paper; HRG & SDS: Advisors in the study; AF, VSN & FS: Analyzed and interpreted the data; FA: reviewing & editing of the manuscript; MG: Conceptualization, designed the experiments, revised, and finalized the manuscript. We confirm that the manuscript has been read and approved by all named authors.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The experimental protocols were established approved by the ethics committee of Mazandaran University of Medical Sciences (Ethical ID: IR.MAZUMC.REC.1399.658). All methods were carried out in the accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Ballén V, Gabasa Y, Ratia C, Ortega R, Tejero M, Soto S (2021) Antibiotic resistance and virulence profiles of *Klebsiella pneumoniae* strains isolated from different clinical sources. Front Cell Infect Microbiol 11:738223

Bina M, Pournajaf A, Mirkalantari S, Talebi M, Irajian G (2015) Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* isolated from the clinical samples by the phenotypic and genotypic methods. Iran J Pathol 10:199–203

Calhau V, Boaventura L, Ribeiro G, Mendonça N, da Silva GJ (2014) Molecular characterization of *Klebsiella pneumoniae* isolated from renal transplanted patients: virulence markers, extended-spectrum β-lactamases, and genetic relatedness. Diagn Microbiol Infect Dis 79:393–395

Chetri S, Bhowmik D, Paul D, Pandey P, Chanda DD, Chakravarty A, Bora D, Bhattacharjee A (2019) AcrAB-TolC efflux pump system plays a role in carbapenem non-susceptibility in *Escherichia coli*. BMC Microbiol 19:210

CLSI supplement M100. Clinical and Laboratory Standards Institute; 2021.

Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, Kassis-Chikhani N, Arlet G, Decré D (2014) Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of *Klebsiella pneumoniae*. J Clin Microbiol 52:4377–4380

Derakhshan S, Najar Peerayeh S, Bakhshi B (2016) Association between presence of virulence genes and antibiotic resistance in clinical *Klebsiella pneumoniae* isolates. Lab Med 47:306–311

Doorduijn DJ, Rooijakkers SH, van Schaik W, Bardoel BW (2016) Complement resistance mechanisms of *Klebsiella pneumoniae*. Immunobiology 221:1102–1109

Ferreira RL, da Silva B, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MC, da Silva EM, Freire CC, Cunha AF, Pranchevicius MC (2019) High prevalence of multidrug-resistant *Klebsiella pneumoniae* harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. Front Microbiol 9:3198

Hall GS (1995) Enterobacteriaceae. In: Mahon CR, Lehman D, Manuselis G (eds) Textbook of diagnostic microbiology, 3rd edn. Saunders Company, New York, pp 464–514

Heiden SE, Hübner NO, Bohnert JA, Heidecke CD, Kramer A, Balau V, Gierer W, Schaefer S, Eckmanns T, Gatermann S, Eger E (2020) A *Klebsiella pneumoniae* ST307 outbreak clone from Germany demonstrates features of extensive drug resistance, hypermucoviscosity, and enhanced iron acquisition. Genome Med 12:1–5

Imai K, Ishibashi N, Kodana M, Tarumoto N, Sakai J, Kawamura T, Takeuchi S, Taji Y, Ebihara Y, Ikebuchi K, Murakami T (2019) Clinical characteristics in blood stream infections caused by *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae*: a comparative study, Japan. BMC Infect Dis 19:946

Imtiaz W, Syed Z, Rafaque Z, Andrews SC, Dasti JI (2021) Analysis of antibiotic resistance and virulence traits (genetic and phenotypic) in *Klebsiella pneumoniae* clinical isolates from Pakistan: identification of significant levels of carbapenem and colistin resistance. Infect Drug Resist 14:227–240

Jian-Li W, Yuan-Yuan S, Shou-Yu G, Fei-Fei D, Jia-Yu Y, Xue-Hua W, Yong-Feng Z, Shi-Jin J, Zhi-Jing X (2017) Serotype and virulence genes of *Klebsiella pneumoniae* isolated from mink and its pathogenesis in mice and mink. Sci Rep 7:1–7

Karaman E, Çiçek AÇ, Şemen V, Şaban Beriş F (2024) Characterization of resistance genes and replicon typing in carbapenem-resistant *Klebsiella pneumoniae* strains. Ann Clin Microbiol Antimicrob 23(1):19

Kundu J, Kansal S, Rathore S, Kaundal M, Angrup A, Biswal M, Walia K, Ray P (2022) Evaluation of ERIC-PCR and MALDI-TOF as typing tools for multidrug resistant *Klebsiella pneumoniae* clinical isolates from a tertiary care center in India. PLoS ONE 17(11):e0271652

Lee CR, Lee JH, Park KS, Jeon JH, Kim YB, Cha CJ, Jeong BC, Lee SH (2023) Antimicrobial resistance surveillance of multidrug-resistant *Klebsiella pneumoniae* in clinical settings: advances and future perspectives. Clin Microbiol Rev 36(2):e00014–23

Lerminiaux NA, Cameron AD (2019) Horizontal transfer of antibiotic resistance genes in clinical environments. Can J Microbiol 65:34–44

Lin TL, Yang FL, Ren CT, Pan YJ, Liao KS, Tu IF, Chang YP, Cheng YY, Wu CY, Wu SH, Wang JT (2022) Development of *Klebsiella pneumoniae* capsule polysaccharide-conjugated vaccine candidates using phage depolymerases. Front Immunol 13:843183

Lin ZW, Zheng JX, Bai B, Xu GJ, Lin FJ, Chen Z, Sun X, Qu D, Yu ZJ, Deng QW (2020) Characteristics of hypervirulent *Klebsiella pneumoniae*: does low expression of rmpA contribute to the absence of hypervirulence?. Front Microbiol 11:436.

Machado E, Coque TM, Cantón R, Baquero F, Sousa JC, Peixe L, Portuguese Resistance Study Group (2006) Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1-producing Enterobacteriaceae strains containing the *aac (6')-Ib-cr* gene, which encodes an aminoglycoside-and fluoroquinolone-modifying enzyme. Antimicrob Agents Chemother 50:3220–3221

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18(3):268–281

Mirzaie A, Ranjbar R (2021) Antibiotic resistance, virulence-associated genes analysis and molecular typing of *Klebsiella pneumoniae* strains recovered from clinical samples. AMB Express 11:122

Moftian N, Rezaei-Hachesu P, Arab-Zozani M, Samad-Soltani T, Esfandiari A, Tabib MS, Mirnia K (2023) Prevalence of gram-negative bacteria and their antibiotic resistance in neonatal sepsis in Iran: a systematic review and meta-analysis. BMC Infect Dis 23(1):534

Nasehi L, Shahcheraghi F, Nikbin VS, Nematzadeh S (2010) PER, CTX-M, TEM and SHV Beta-lactamases in clinical isolates of *Klebsiella pneumoniae* isolated from Tehran, Iran. Iran J Basic Med Sci 13:111–118

Podschun R, Ullmann U (1998) *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 11:589–603

Qiu Y, Lin D, Xu Y, Cheng Y, Wang F, Zhu Q, Zhu C, Wan C, Zhu Y, Tong J, Li R (2021) Invasive *Klebsiella pneumoniae* infections in community-settings and healthcare settings. Infect Drug Resist 14:2647–2656

Ranjbar R, Fatahian Kelishadrokhi A, Chehelgerdi M (2019) Molecular characterization, serotypes and phenotypic and genotypic evaluation of antibiotic resistance of the *Klebsiella pneumoniae* strains isolated from different types of hospital-acquired infections. Infect Drug Resist 603–611

Riwu KHP, Effendi MH, Rantam FA, Khairullah AR, Widodo A (2022) A review: virulence factors of *Klebsiella pneumoniae* as an emerging infection in the food chain. Vet World 15(9):2172–2179

Saki H, Haddadi A, Shavandi M (2019) Integron-associated antibiotic resistance in clinical isolates of *Klebsiella* from Karaj. J Arak Univ Med Sci 21:48–57

Sanikhani R, Moeinirad M, Solgi H, Hadadi A, Shahcheraghi F, Badmasti F (2021) The face of hypervirulent *Klebsiella pneumoniae* isolated from clinical samples of two Iranian teaching hospitals. Ann Clin Microbiol Antimicrob 20:58

Sedighi P, Zarei O, Karimi K, Taheri M, Karami P, Shokoohizadeh L (2020) Molecular typing of *Klebsiella pneumoniae* clinical isolates by Enterobacterial repetitive intergenic consensus polymerase chain reaction. Int J Microbiol 8894727

Shoeib N, Fereshteh S, Mehdi FM, Sadat NV, Leila N (2011) Molecular characterization of CTX-M β-lactamases among *Klebsiella pneumoniae* isolated from patients at Tehran hospitals. Indian J Med Microbiol 29:254–257
Shrivastava SR, Shrivastava PS, Ramasamy J (2018) World Health Organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. J Med Soc 32:76–77
Struve C, Krogfelt KA (2003) Role of capsule in *Klebsiella pneumoniae* virulence: lack of correlation between in vitro and in vivo studies. FEMS Microbiol Lett 218:149–154
Tapia-Pastrana G, Rojas-Bautista M, Hernández-Pérez P, Santiago-Martínez O, Gómez-Rodríguez LC, Terrazas-Luna VM, Montes-Yedra J, Bautista-Avendaño AA, García-López ES, Leon-Sicairosm N, Angulo-Zamudio UA (2024) Virulence genes, antimicrobial resistance profile, phylotyping and pathotyping of diarrheagenic Escherichia coli isolated from children in Southwest Mexico. PLoS ONE 19(3):e0300304
Türkel İ, Yıldırım T, Yazgan B, Bilgin M, Başbulut E (2018) Relationship between antibiotic resistance, efflux pumps, and biofilm formation in extended-spectrum β-lactamase producing *Klebsiella pneumoniae*. J Chemother 30:354–363
Vaez H, Sahebkar A, Khademi F (2019) Carbapenem-resistant *Klebsiella pneumoniae* in Iran: a systematic review and meta-analysis. J Chemother 31:1–8
Wasfi R, Elkhatib WF, Ashour HM (2016) Molecular typing and virulence analysis of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from Egyptian hospitals. Sci Rep 6:1–1
Wyres KL, Lam MM, Holt KE (2020) Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol 18:344–359

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