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Molecular Characterization of Beta-Lactamase-Encoding Genes and Associated Antimicrobial Resistance in Clinical Isolates of *Klebsiella pneumoniae* from Zanjan, Iran

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Abstract

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is a major cause of hospital-acquired infections, where treatment options are restricted due to drug-resistant strains, including those producing extended-spectrum beta-lactamases (ESBLs) and carbapenemases.

Objectives: This study focuses on the molecular identification of beta-lactamase and carbapenemase genes in *K. pneumoniae* strains from Valiasr Hospital, Zanjan, to enhance infection control and antimicrobial strategies.

Methods: In this cross-sectional study, 50 *K. pneumoniae* clinical isolates were obtained from blood, urine, and wound samples of ICU patients in 2024. Phenotypic identification and antimicrobial susceptibility testing were performed using the Kirby-Bauer disk diffusion method. Genomic DNA extraction was performed using the phenol-chloroform method, followed by polymerase chain reaction (PCR) to identify resistance genes (bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{IMP}, bla_{VIM}). The PCR products were analyzed via agarose gel electrophoresis, with data processed using SPSS version 26.

Results: The mean age of the patients was 58.90 years, and the isolates exhibited high resistance rates to ampicillin (90%), piperacillin (78%), and cefotaxime (72%). Genotypic testing indicated ESBL gene frequencies: bla_{TEM} (46%), bla_{SHV} (44%), and bla_{CTX-M} (40%), along with carbapenemase genes bla_{VIM} and bla_{IMP} in 26% and 22% of isolates, respectively. A significant link was found between the bla_{TEM} gene and urinary isolates ($P = 0.037$). Furthermore, the presence of the bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes was associated with MDR, XDR, and PDR phenotypes ($P < 0.05$), with 100% of MDR isolates containing all three genes.

Conclusion: The study found a high prevalence of ESBL genes linked to multidrug resistance patterns among *K. pneumoniae* isolates in ICUs. The presence of carbapenemase genes (bla_{IMP} and bla_{VIM}) indicates a shift toward carbapenem resistance, underscoring the urgent need for better antibiotic surveillance and infection control in these settings.

Keywords: *Klebsiella pneumoniae*, Beta-Lactamase, Cross Infection, Drug Resistance

1. Background

Antibiotics play a major role in human survival and quality of life by treating and eradicating infectious diseases.[1] However, the overuse of antibiotics has led to an increase in drug resistance in most bacterial strains, even against a wide spectrum of antibiotics.[2] Carl Friedlander discovered *Klebsiella pneumoniae* (*K. pneumoniae*) in 1882 as a causative agent of pneumonia in patients; it is a rod-shaped, encapsulated, facultative anaerobic, non-spore-forming, and opportunistic pathogen belonging to the family Enterobacteriaceae.[3,4] The Gram-negative bacterium, it is known as a causative agent of bacteremia, pneumonia, and urinary tract infections. Furthermore, the misuse of antimicrobial drugs and the proliferation of antibiotic-resistant bacteria have raised serious concerns in hospital settings. Increasing mortality rates, neonatal sepsis, and drug-resistant infections are among the worldwide health problems exacerbated by this issue.[5,6] The emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) strains has created significant challenges in the field of infection control and achieving effective treatments in hospital settings.[7] However, the MDR strains of this bacterium are becoming more prevalent due to selective pressure resulting from widespread antibiotic use, as well as the horizontal transfer of resistance genes, particularly through plasmids, reducing the efficacy of antibiotic treatments by employing strategies such as the synthesis of drug-inactivating enzymes, modification of antibiotic target sites, increased activity of efflux pumps, and decreased membrane permeability.[8] The ability of harmful bacteria to resist drugs such as beta-lactams is known as antimicrobial resistance and is mainly caused by enzymes such as

extended-spectrum beta-lactamases (ESBLs), which inactivate these antibiotics.[9] Most Gram-negative organisms have been recognized as ESBL producers, and over the past 20 years, ESBL-producing *K. pneumoniae* strains have been associated with multiple outbreaks of nosocomial infections.[1,10] ESBLs, which were first identified in Germany in 1983, specifically target penicillins and broad-spectrum cephalosporins, predominantly found in Enterobacteriaceae, particularly *K. pneumoniae*, with common classifications including Temoniera (TEM), Sulfhydryl Variable (SHV), and Cefotaximase (CTX-M).[11,12] Furthermore, bla_{IMP} and bla_{VIM} are integron-bound genes located on either the chromosome or plasmids, that are capable of degrading carbapenems, penicillins, cephalosporins, and monobactams.[13]

2. Objectives

The global increase in MDR *K. pneumoniae* emphasizes the necessity of developing innovative treatments, such as novel vaccines, antibiotics, and artificial intelligence-based methods, as well as strengthening international cooperation and antimicrobial stewardship.[14] Hence, the aim of this study was to investigate the antibiotic resistance pattern and the prevalence of bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{IMP}, and bla_{VIM} genes in *K. pneumoniae* isolates obtained from clinical specimens in Zanjan, Iran.

3. Methods

3.1. Study Design, Research Setting, and Sampling

During 2024, a total of 50 presumptive *K. pneumoniae* isolates were investigated at Valiasr Hospital in Zanjan, Iran. Clinical specimens, including blood, wound exudates, and urine, were collected from patients admitted to the intensive care unit (ICU) who developed nosocomial infections 48 to72 hours post-admission. Only isolates previously identified as *K. pneumoniae* were eligible for inclusion, and to prevent data redundancy, only the initial isolate from each patient was evaluated. Exclusion criteria comprised the absence of informed consent, compromised sample integrity, incomplete documentation, contaminated cultures, or community-acquired infections were excluded. Definitive identification of the remaining isolates was subsequently achieved using standard microbiological assays.

3.2. Isolation and Identification of Bacteria

For isolation of *K. pneumoniae*, clinical specimens were inoculated on blood and MacConkey agar plates and incubated at 37 °C for 24 to 48 hours. Initially growing colonies were Gram stained (Gram-negative bacilli or coccobacilli). Biochemical testing then confirmed the *Klebsiella* genus with positive results for catalase and citrate, negative results for oxidase and indole, a positive urease reaction and characteristic MR-VP profiles. Finally, the chosen strains were suspended in Trypticase Soy Broth with 30% glycerol and stored at -70 °C for later analysis.

3.3. Antibiotic Susceptibility Testing Using the Disk Diffusion Method

Antibiotic susceptibility testing was performed using the disk diffusion (Kirby-Bauer) method on Mueller-Hinton agar. The tested antibiotics included gentamicin (10 µg), ampicillin/sulbactam (20 µg), ciprofloxacin (5 µg), ampicillin (10 µg), co-trimoxazole (1.25/25 µg), cefotaxime (30 µg), piperacillin (100 µg), cefepime (30 µg), imipenem (10 µg), aztreonam (30 µg), levofloxacin (5 µg), amikacin (30 µg), and tetracycline (30 µg). A bacterial suspension equivalent to a 0.5 McFarland standard (10^8 CFU/ml) was prepared, inoculated onto the medium, and incubated at 37 °C for 18 hours. Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (2021 edition) using *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 as quality control strains. Finally, the isolates were categorized into three groups based on their resistance profiles: MDR, XDR, and PDR. All culture media were obtained from Merck (Germany), and all antibiotics from Padten Teb (Iran).

3.4. Polymerase Chain Reaction (PCR)

3.4.1. Genomic DNA Extraction and Quality Assessment

The phenol-chloroform technique was used to extract genomic DNA, and a NanoDrop spectrophotometer was used to evaluate DNA concentration and purity based on the absorbance ratio at 260/280 nm. Samples with ratios in the acceptable range of 1.8 to 1.9 were selected for further analysis.

Table 1. Sequence of Primers Used to Detect the Genes Under Study

Genes	Primer sequence (5′→ 3′)	Annealing temperature (°C)	Band size (Bp)
bla_{CTX-M}	Forward: TTTGCGAGTGCAGTACCAGTAA Reverse: CTCCCCTGCCGGTTTTATC	56	519
bla_{TEM}	Forward: ATGAGTATTCAACATTTCCG Reverse: CTGACAGTTACCAATGCTTA	58	867
bla_{SHV}	Forward: TTAACTCCCTGTTAGCCA Reverse: GATTTGCTGATTTCGCCC	55	796
bla_{IMP}	Forward: GGAATAGAGTGGCTTAACTCTC Reverse: CCAAACCACTAGGTTATCT	56	190
bla_{VIM}	Forward: CAAGTCCGTTAGCCCATTCC Reverse: GGCACAACCACCGTATAGCAC	60	539

3.4.2. Target Genes and PCR Thermal Cycling Conditions

Five beta-lactamase genes were examined in this study: bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{IMP}, and bla_{VIM} (primer sequences and properties are shown in Table 1). PCR was carried out in a final volume of 25 µl that included 3 µl of template DNA, 7.5 µl of nuclease-free water, 1 µl of each primer (25 pmol), and 12.5 µl of commercial master mix (PaqGen, Iran). The PCR thermal cycling conditions were as follows: 5 minutes of initial denaturation at 94 °C, 35 cycles of denaturation at 94 °C for 45 seconds, 45 seconds of annealing at each gene's ideal temperature, 60 seconds of extension at 72 °C, and 5 minutes of final extension at 72 °C.

3.4.3. Electrophoresis and Interpretation of Results

Following electrophoresis on a 1% agarose gel, the PCR results were stained with Safe Stain, and the resultant bands were examined and recorded under ultraviolet light.

3.5. Statistical Analysis

IBM SPSS Statistics version 26 was used for all statistical analyses. Chi-square (χ^2) tests were employed to ascertain the clonal association between MDR, XDR, and PDR strains as well as the relationship between the common types of *K. pneumoniae* and their antibiotic resistance patterns.

4. Results

4.1. Demographic Characteristics of Patients and Clinical Isolates

The study examined 50 isolates of *K. pneumoniae*, revealing a mean patient age of 58.90 years, with a range of 29 to 86 years and a standard deviation of 14.44. The majority of cases (42%, 21 cases) were found in patients aged 61 to 80 years. The isolates were predominantly associated with urinary infections (68%), followed by blood (20%) and wound secretions (12%), indicating that urinary tract infections are a common issue within this population. The gender distribution showed a higher incidence in males (62%, 31 cases) compared to females (38%, 19 cases), a trend consistent across most age groups, except for those over 81 where cases were few. In the 41 to 60 age group, 20 cases included 11 males and 9 females. The lowest infection rate was noted among individuals aged 29 to 40, with only 7 cases (14%), of which 6 were male, and 1 was female.

Table 2. Age Distribution of the Studied Patients

Age group (years)	n (%)	Female n (%)	Male n (%)
29-40	7(14%)	1 (5.26%)	6 (19.35%)
41 – 60	20 (40%)	9 (47.37%)	11 (35.48%)
61 – 80	21 (42%)	8 (42.11%)	13 (41.96%)
> 81	2 (4%)	1 (5.26%)	1 (3.23%)
Total	**50 (100%)**	**19 (100%)**	**31 (100%)**

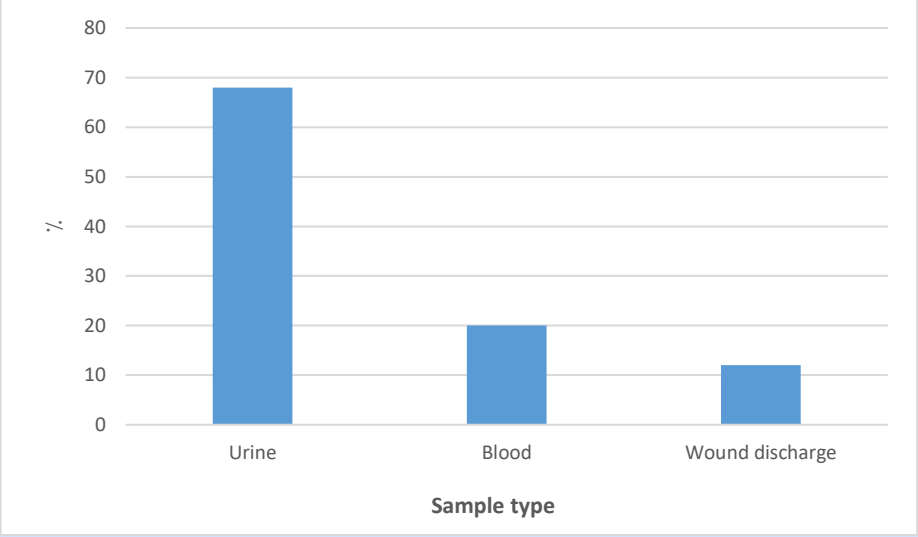

Figure 1. Percentage of Clinical Specimens in *K. Pneumoniae* Isolates.

4.2. Antibiotic Susceptibility Pattern

Based on the data in Table 3, the antibiotic resistance profile of 50 isolates reveals significant multidrug resistance. Resistance rates for beta-lactam antibiotics are particularly high, with ampicillin at 90%, piperacillin at 78%, and cefotaxime at 72%, indicating extensive beta-lactamase enzyme production among these bacteria. Broad-spectrum antibiotics like cefepime (58% resistance) and aztreonam (60% resistance) also show high ineffectiveness. Furthermore, aminoglycoside and fluoroquinolone antibiotics display notable resistance, with gentamicin at 62% and ciprofloxacin at 66%. In contrast, imipenem

remains highly effective, with 76% susceptibility and only 6% resistance, establishing carbapenems as the last line of defense against these infections. Levofloxacin (50% susceptible) and co-trimoxazole (60% susceptible) are alternatives, but resistance to them is prevalent as well.

Table 3. Antimicrobial Susceptibility Profile of *K. Pneumoniae* Isolates

Antibiotic	Resistance n (%)	Intermediate n (%)	Sensitive n (%)
Tetracycline	27 (54)	9 (18)	14 (28)
Amikacin	27 (54)	13 (26)	10 (20)
Levofloxacin	11 (22)	14 (28)	25 (50)
Aztreonam	30 (60)	5 (10)	15 (30)
Imipenem	3 (6)	9 (18)	38 (76)
Cefepime	29 (58)	7 (14)	14 (28)
Piperacillin	39 (78)	8 (16)	3 (6)
Cefotaxime	36 (72)	8 (16)	6 (12)
Co-trimoxazole	18 (36)	2 (4)	30 (60)
Ampicillin	45 (90)	0 (0)	5 (10)
Ciprofloxacin	33 (66)	10 (20)	7 (14)
Ampicillin / Sulbactam	27 (54)	5 (10)	16 (36)
Gentamicin	31 (62)	15 (30)	4 (8)

The study found no consistent or significant association between antibiotic resistance, demographic variables, and sample types, as indicated by *P*-values predominantly greater than 0.05. This suggests that age, gender, and the type of sample (urine, blood, or wound secretion) had no substantial impact on resistance patterns for most antibiotics. However, piperacillin resistance was a notable exception, showing a significant association with sample type ($P = 0.009$), hinting at potential differences in resistance across urine, blood, and wound secretion samples (Table 4).

4.3. Phenotypic Patterns of Resistance (MDR, XDR and PDR)

Based on findings in Table 5, a significant pattern of multidrug resistance was identified among *K. pneumoniae* isolates. Specifically, 68% of the isolates were categorized as MDR, 58% as XDR, and 49% as PDR. This trend illustrates a concerning progression (MDR > XDR > PDR), indicating a severe crisis in antibiotic resistance. Age distribution revealed that the highest occurrences in these groups were in individuals aged 41-60 years (41.86% for MDR), 61-89 years (44.83% for XDR), and 41-60 years (45% for PDR). The male proportion across the groups was 60.47% (MDR), 61.97% (XDR), and 75% (PDR), with urine samples reflecting 69.77%, 62.07%, and 65% of these isolates, respectively. Nevertheless, statistical analysis indicated no significant correlation between resistance patterns (MDR, XDR, PDR) and the variables of age, gender, or type of clinical specimen ($P > 0.05$).

4.4. Molecular Identification of Resistance Genes

The antibiotic resistance genes of the ESBLs bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV} and the metallo-beta-lactamase genes bla_{IMP} and bla_{VIM} were examined using specific primers and PCR method. The profile of the beta-lactamase genes from the present study is shown in Figure 2. The results of the analysis of antibiotic resistance genes in the studied population showed that among the genes associated with antibiotic resistance, the bla_{SHV} (44%), bla_{TEM} (46%) and $bla_{CTX\text{-}M}$ (40%) genes were more abundant than the carbapenem resistance genes including bla_{VIM} (26%) and bla_{IMP} (22%).

Based on the presented data, a notable distribution pattern of pathogen genes across age groups, gender, and sample types has been identified. The highest gene segregation frequency was found in individuals aged 61 to 80 years, with males showing the greatest percentage of the genes studied. Urine samples were predominantly significant for gene isolation, with 40% of the $bla_{CTX\text{-}M}$ gene isolated from the 41-60 age group, 60% from men, and 65% from urine samples. The bla_{TEM} gene showed 47.83% isolation among the 61-80 age group, with 65.22% from men and 82.61% from urine samples. For the bla_{SHV} gene, 45.45% isolation was from the 61-80 age group, also reporting 63.64% from men and urine samples. The bla_{IMP} gene isolation was noted at 36.36%

Table 4. Antibiotic Resistance in *K. pneumoniae* Isolates by Age Group, Specimen Type, and Gender

Antibiotics	Result	Total	Gender, n (%)			Sample type, n (%)				Age, n (%)				
			Female	Male	*P*	Urine	Blood	Wound discharge	*P*	29 to 40	41 to 60	61 to 80	More than 81	*P*
Tetracycline	Sensitive	14	7 (50)	7 (50)		8 (57.14)	3 (21.43)	3 (21.43)		3(21.43)	6(42.86)	5 (35.71)	0	
	Intermediate	9	4 (44.44)	5(55.56)	0.403	8 (88.89)	1 (11.11)	0	0.493	2(22.22)	4(44.44)	2 (22.22)	1 (11.11)	0.540
	Resistant	27	8 (29.63)	19 (70.37)		18 (66.67)	6 (22.22)	3 (11.11)		2 (7.41)	10 (37.04)	14 (51.85)	1 (3.70)	
Amikacin	Sensitive	10	3 (30)	7 (70)		4 (40)	3 (30)	3(30)		2 (20)	3 (30)	4 (40)	1 (10)	
	Intermediate	13	6 (46.15)	7 (53.85)	0.723	10 (76.92)	2 (15.38)	1 (7.69)	0.240	1(7.69)	6 (46.15)	5 (38.46)	1 (7.69)	0.703
	Resistant	27	10 (37.04)	17 (62.96)		20 (74.07)	5 (18.52)	2 (7.41)		4(14.81)	11 (40.74)	12 (44.44)	0	
Levofloxacin	Sensitive	25	10 (40)	15 (60)		16 (64)	4 (16)	5 (20)		4 (16)	8 (32)	13 (52)	0	
	Intermediate	14	7 (50)	7 (50)	0.225	11 (78.57)	2 (14.29)	1 (7.14)	0.272	2 (14.29)	6 (42.86)	5 (35.71)	1 (7.14)	0.576
	Resistant	11	2 (18.18)	9 (81.82)		7 (63.64)	4 (36.36)	0		1(9.09)	6 (54.55)	3 (27.27)	1 (9.09)	
Aztreonam	Sensitive	15	5 (33.33)	10 (66.67)		13 (86.67)	0	2 (13.33)		3(20)	7 (46.67)	5 (33.33)	0	
	Intermediate	5	4 (80)	1 (20)	0.125	2 (40)	3 (60)	0	0.055	0	3 (60)	2(40)	0	0.278
	Resistant	30	10 (33.33)	20 (66.67)		19 (63.33)	7 (23.34)	4 (13.33)		4(13.33)	10 (33.33)	14 (46.67)	2 (6.67)	
Imipenem	Sensitive	38	16 (42.11)	22 (57.89)		25 (65.79)	8 (21.05)	5 (13.16)		6(15.79)	15 (39.47)	15 (39.47)	2 (5.26)	
	Intermediate	9	2 (22.22)	7 (77.78)	0.535	6 (66.67)	2 (22.22)	1 (11.11)	0.821	1(11.11)	3 (33.33)	5 (55.56)	0	0.542
	Resistant	3	1 (33.33)	2 (66.67)		3(100)	0	0		0	2 (66.67)	1 (33.33)	0	
Cefepime	Sensitive	14	5 (35.71)	9 (64.29)		11(78.57)	1(7.14)	2(14.29)		2 (14.29)	5 (35.71)	7 (50)	0	
	Intermediate	7	2 (28.57)	5 (71.43)	0.804	4(57.14)	2(28.57)	1(14.29)	0.699	2 (28.57)	1 (14.29)	4 (57.14)	0	0.520
	Resistant	29	12 (41.38)	17 (58.62)		19(65.52)	7(24.14)	3(10.34)		3 (10.34)	14(48.28)	10 (34.48)	2 (6.90)	
Piperacilin	Sensitive	3	2 (66.67)	1 (33.33)		0	2(66.67)	1(33.33)		1 (33.33)	2 (66.67)	0	0	
	Intermediate	8	3 (37.5)	5 (62.5)	0.571	4(50)	1(12.5)	3(37.5)	0.009	1 (125)	3 (37.5)	4 (40)	0	0.732
	Resistant	39	14 (35.90)	25 (64.10)		30(76.92)	7(17.95)	2(5.13)		5(12.82)	15(38.46)	17(43.59)	2(5.13)	
Cefotaxime	Sensitive	6	4 (66.67)	2 (33.33)		3(50)	2(33.33)	1(16.67)		0	3(50)	3(50)	0	
	Intermediate	8	3 (37.5)	5 (62.5)	0.297	6(75)	2(25)	0	0.669	2(25)	2(25)	4(50)	0	0.794
	Resistant	36	12 (33.33)	24 (66.67)		25(69.44)	6(16.67)	5(13.89)		5(13.89)	15(41.67)	14(38.89)	2(5.56)	
Co-trimoxazol	Sensitive	30	11 (36.67)	19 (63.33)		19(63.33)	7(23.33)	4(13.33)		5(16.67)	11(36.67)	14(46.67)	0	
	Intermediate	2	1 (50)	1 (50)	0.927	2(100)	0	0	0.843	0	2(100)	0	0	0.941
	Resistant	18	7 (38.89)	11 (61.11)		13(72.22)	3(16.67)	2(11.11)		2(11.11)	7(38.89)	7(38.89)	2(11.11)	
Ampicillin	Sensitive	5	2 (40)	3 (60)		2(40)	2(40)	1(20)		1(20)	2(40)	2(40)	0	
	Intermediate	0	0	0	0.923	0	0	0	0.359	0	0	0	0	0.231
	Resistant	45	17 (37.78)	28 (62.22)		32(71.11)	8(17.78)	5(11.11)		6(13.33)	18(40)	19(42.22)	2(4.44)	
Ciprofloxacin	Sensitive	7	3 (42.86)	4 (57.14)		3(42.86)	1(14.29)	3(42.86)		1(14.29)	2(28.57)	3(42.86)	1(14.29)	
	Intermediate	10	5 (50)	5 (50)	0.611	8(80)	1(10)	1(10)	0.080	1(10)	3(30)	6(60)	0	0.722
	Resistant	33	11 (33.33)	22 (66.67)		23(69.70)	8(24.24)	2(6.06)		5(15.15)	15(45.45)	12(36.36)	1(3.03)	
Ampicillin / Sulbactam	Sensitive	18	8 (44.44)	10 (55.56)		11(61.11)	3(16.67)	4(22.22)		1(5.56)	8(44.44)	9(50)	0	
	Intermediate	5	2 (40)	3 (60)	0.750	5(100)	0	0	0.277	1(20)	2(40)	1(20)	1(20)	0.264
	Resistant	27	9 (33.33)	18 (66.67)		18(66.67)	7(25.93)	2(7.41)		5(18.52)	10(37.04)	11(40.74)	1(3.70)	
Gentamicin	Sensitive	4	2 (50)	2 (50)		3(75)	1(25)	0		0	0	4(100)	0	
	Intermediate	15	4 (26.67)	11 (73.33)	0.531	12(80)	1(6.67)	2(13.33)	0.561	2(13.33)	5(33.33)	7(46.67)	1(6.67)	0.455
	Resistant	31	13 (41.94)	18 (58.06)		19(61.29)	8(25.81)	4(12.90)		5(16.13)	15(48.39)	10(32.26)	1(3.23)	

Table 5. Frequency of Resistance Phenotypes (MDR, XDR, PDR) in Clinical Samples

		MDR	*P*	XDR	*P*	PDR	*P*
Age	29 to 40	6 (13.95)	0.934	3 (10.34)	0.106	2 (10)	0.395
	41 to 60	18 (41.86)		12 (41.38)		8 (40)	
	61 to 80	17 (39.53)		13 (44.83)		9 (45)	
	More than 81	2 (4.65)		1 (3.45)		1 (5)	
Genus	Female	17 (39.53)	0.579	9 (31.03)	0.233	5 (25)	0.122
	Male	26 (60.47)		20 (61.97)		15 (75)	
Type of specimen	Urine	30 (69.77)	0.344	18 (62.07)	0.567	13 (65)	0.751
	Blood	9 (20.93)		7 (24.14)		5 (25)	
	Wound discharge	4 (9.30)		4 (13.79)		2 (10)	
Total		43		29		20	

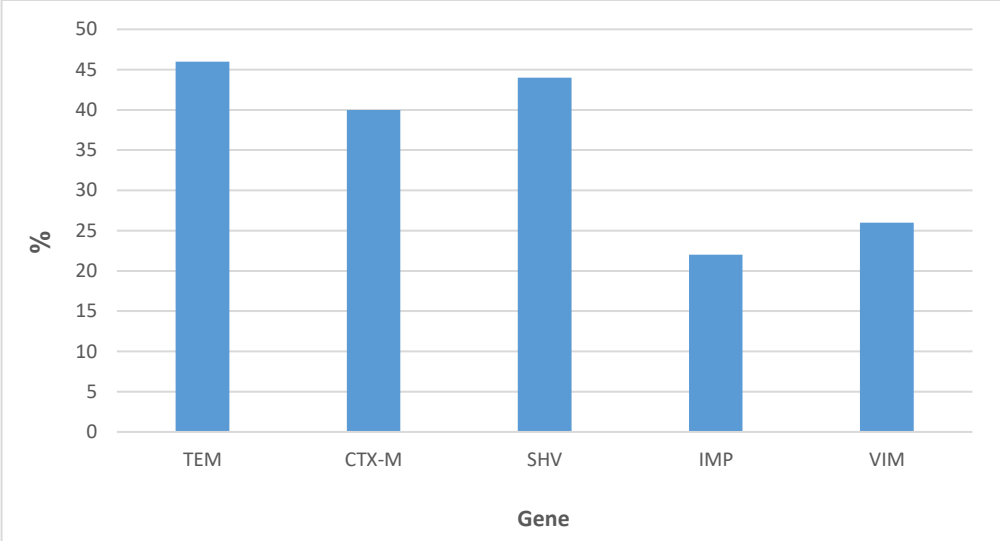

Figure 2. Profile of Beta-lactamase Genes in the Studied *K. Pneumoniae* Isolates.

from the 29-40 and 61-80 age groups, with a significant male presence (81.82%) and 63.64% from urine samples. The bla_{VIM} gene displayed a 46.15% isolation rate among the 61-80 age group, 69.23% from men, and 53.85% from urine samples. Statistical analysis indicated a significant association (P = 0.037) between the bla_{TEM} gene's presence and urine sample type, highlighting its dependence on urine samples.

4.5. Relationship between Resistance Genes and Antibiotic Resistance

The study analyzed the correlation between antibiotic resistance and specific genes, highlighting significant relationships. Resistance to various antibiotics, including tetracycline, aztreonam, imipenem, cefepime, cefotaxime, and cotrimoxazole, was notably linked to the bla_{TEM} gene. The bla_{CTX-M} gene was associated with resistance to aztreonam, cefepime, cefotaxime, and gentamicin, while the bla_{SHV} gene presented a broader resistance profile covering aztreonam, cefepime, piperacillin, cefotaxime, ampicillin, ciprofloxacin, and gentamicin. Additionally, resistance to gentamicin was associated with the bla_{IMP} gene. All correlations were statistically significant (P < 0.05). The findings underscore the critical role of beta-lactamase genes in multidrug resistance, with the bla_{SHV} gene conferring the most extensive resistance across four major antibiotic classes, whereas the bla_{TEM} gene provided resistance against carbapenems, monobactams, cephalosporins, and some non-beta-lactam antibiotics. The bla_{CTX-M} gene exhibited distinct resistance patterns against monobactams and newer generations of cephalosporins and aminoglycosides (Table 7).

4.6. Association between Resistance Genes and MDR, XDR and PDR Phenotypes

Analysis of the association between virulence genes and antibiotic resistance factors with MDR, XDR, and PDR phenotypes in *K. pneumoniae* isolates revealed highly significant statistical relationships. Genes encoding ESBLs and metallo-beta-lactamases demonstrated a strong correlation. Specifically, the bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes were significantly associated with all three MDR, XDR, and PDR patterns (P < 0.05). Notably, the MDR phenotype was observed in 100% of the isolates harboring these three genes, while high rates of XDR (ranging from 82.61% to 90.91%) and PDR (ranging from 60.87% to 65%) were also recorded. This finding clearly confirms the pivotal role of these beta-lactam resistance genes in the development of multidrug resistance in this bacterium. Conversely, no significant association was observed for the bla_{IMP} and bla_{VIM} genes (Table 8).

Table 6. Prevalence of Target Genes by Age, Gender, and Specimen Type

	Age, n (%)					Gender, n (%)			Type of Specimen, n (%)			
	29 to 40	**41 to 60**	**61 to 80**	**More than 81**	***P***	**Female**	**Male**	***P***	**Urine**	**Blood**	**Wound discharge**	***P***
bla$_{CTX-M}$	4(20)	8(40)	7(35)	1(5)	0.269	8(40)	12(60)	0.812	13(65)	4(20)	3(15)	0.863
bla$_{TEM}$	3(13.04)	7(30.43)	11(47.83)	2(8.70)	0.171	8(34.78)	15(65.22)	0.665	19(82.61)	1(4.35)	3(13.04)	0.037
bla$_{SHV}$	4(18.18)	7(31.82)	10(45.45)	1(4.54)	0.144	8(36.36)	14(63.64)	0.833	14(63.64)	5(22.73)	3(13.64)	0.842
bla$_{IMP}$	4(36.36)	3(27.27)	4(36.36)	0	0.498	2(18.18)	9(81.82)	0.125	7(63.64)	3(27.27)	1(9.09)	0.774
bla$_{VIM}$	3(23.08)	4(30.77)	6(46.15)	0	0.796	4(30.77)	9(69.23)	0.532	7(53.85)	4(30.77)	2(15.38)	0.427

Table 7. Association between Antibiotic Resistance and Presence of Target Genes (S / I / R for each antibiotic)

n (%)		Tetracycline	Amikacin	Levofloxacin	Aztreonam	Imipenem	Cefepime	Piperacillin	Cefotaxime	Co-trimoxazole	Ampicillin	Ciprofloxacin	Ampicillin / Sulbactam	Gentamicin
bla$_{TEM}$	**S**	2(8.69)	3(13.04)	9(39.13)	2(8.69)	14(60.87)	3(13.04)	1(4.35)	0	9(39.13)	1(4.35)	2(8.69)	6(26.09)	0
	I	5(21.74)	4(17.39)	8(34.78)	2(8.69)	6(26.09)	2(8.69)	2(8.69)	0	1(4.35)	0(0)	2(8.69)	3(13.04)	6(26.09)
	R	16(69.56)	16(69.56)	6(26.09)	19(82.61)	3(13.04)	18(78.26)	20(86.96)	23(100)	13(56.52)	22(95.65)	19(82.61)	14(60.87)	17(73.91)
	p	0.019	0.125	0.362	0.006	0.042	0.026	0.358	0.000	0.018	0.219	0.069	0.381	0.100
bla$_{CTX-M}$	**S**	5(25)	2(10)	9(45)	1(5)	18(90)	2(10)	1(5)	0	9(45)	1(5)	2(10)	6(30)	0
	I	2(10)	3(15)	4(20)	1(5)	1(5)	2(10)	1(5)	1(5)	0	0	1(5)	2(10)	3(15)
	R	13(65)	15(75)	7(35)	18(90)	1(5)	16(80)	18(90)	19(95)	11(55)	19(95)	17(85)	12(60)	17(85)
	p	0.361	0.051	0.175	0.002	0.132	0.030	0.203	0.011	0.052	0.336	0.051	0.757	0.018
bla$_{SHV}$	**S**	4(18.18)	4(18.18)	9(40.91)	2(9.09)	15(68.18)	0	0	0	11(50)	0	1(4.54)	5(22.73)	1(4.54)
	I	3(13.64)	4(18.18)	7(31.82)	2(9.09)	6(27.27)	3(13.64)	1(4.54)	0	0	0	1(4.54)	2(9.09)	2(9.09)
	R	15(68.18)	14(63.64)	6(27.27)	18(81.82)	1(4.54)	19(86.36)	21(95.45)	22(100)	11(50)	22(100)	20(90.91)	15(68.18)	19(86.36)
	p	0.199	0.435	0.509	0.012	0.312	0.000	0.029	0.000	0.113	0.037	0.004	0.181	0.006
bla$_{IMP}$	**S**	3(27.27)	0	7(63.64)	1(9.09)	9(81.82)	3(27.27)	1(9.09)	0	9(81.82)	1(9.09)	0	2(18.18)	1(9.09)
	I	1(9.09)	2(18.18)	3(27.27)	0	1(9.09)	1(9.09)	0	3(27.27)	1(9.09)	0	3(27.27)	0	0
	R	7(63.64)	9(81.82)	1(9.09)	10(90.91)	1(9.09)	7(63.64)	10(90.91)	8(72.73)	1(9.09)	10(90.91)	8(72.73)	9(81.82)	10(90.91)
	p	0.648	0.075	0.450	0.058	0.635	0.851	0.249	0.245	0.088	0.909	0.295	0.097	0.046
bla$_{VIM}$	**S**	5(38.46)	3(23.08)	7(53.85)	3(23.08)	8(61.54)	2(15.38)	1(7.69)	2(15.38)	9(69.23)	3(23.08)	2(15.38)	3(23.08)	1(7.69)
	I	2(15.38)	1(7.69)	5(38.46)	1(7.69)	4(30.77)	2(15.38)	2(15.38)	3(23.08)	0	0	3(23.08)	1(7.69)	5(38.46)
	R	6(46.15)	9(69.23)	1(7.69)	9(69.23)	1(7.69)	9(69.23)	10(76.92)	8(61.54)	4(30.77)	10(76.92)	8(61.54)	9(69.23)	7(53.85)
	p	0.621	0.212	0.303	0.732	0.340	0.495	0.956	0.611	0.581	0.068	0.923	0.435	0.737

Table 8. Association between Resistance Phenotypes (MDR, XDR, PDR) and Target Genes

	MDR		XDR		PDR	
	n (%)	*P*	n (%)	*P*	n (%)	*P*
bla$_{CTX-M}$	20 (100)	0.020	17 (85)	0.002	13 (65)	0.003
bla$_{TEM}$	23 (100)	0.008	19 (82.61)	0.001	14 (60.87)	0.005
bla$_{SHV}$	22 (100)	0.011	20 (90.91)	0.000	14 (63.64)	0.002
bla$_{IMP}$	10 (90.91)	0.595	8 (72.73)	0.262	6 (54.55)	0.265
bla$_{VIM}$	10 (76.92)	0.273	7 (53.85)	0.724	5 (38.46)	0.895

5. Discussion

ESBLs are enzymes produced by certain Gram-negative bacteria, particularly *K. pneumoniae*, that confer resistance to a broad spectrum of beta-lactam antibiotics, including penicillins and cephalosporins. Resistance in these bacteria is primarily mediated by the bla gene family, the most prominent of which includes *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M.}$[15] At the molecular level, these genes are frequently located on mobile genetic elements, particularly plasmids, facilitating rapid horizontal transfer between strains and even across bacterial species. This horizontal transfer can lead to the co-accumulation of multiple resistance genes within a single isolate, ultimately resulting in MDR, XDR, and PDR phenotypes.[16,17] In the present study, the frequencies of the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes were 46%, 44%, and 40%, respectively, indicating the predominant role of ESBLs in beta-lactam resistance. Furthermore, the high prevalence of *bla*$_{CTX-M}$ and *bla*$_{SHV}$ underscores their role in the regional dissemination of ESBLs and the establishment of this mechanism within the hospital environment. These findings correlate with the high prevalence of MDR (68%), XDR (58%), and PDR (49%), as well as widespread resistance to cephalosporins, demonstrating that the plasmid-mediated dissemination of these genes plays a pivotal role in increasing the resistance burden in the ICU. Compared with other studies, Maleki et al.[18] reported 44.9% of isolates as MDR, which was lower than the 68% observed in the present study, whereas the frequencies of *bla*$_{CTX-M}$ (92%) and *bla*$_{TEM}$ (76%) in that study were higher than our results. Mansagna et al.[19] also reported the prevalence of *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ as 60%, 32.5%, and 7.5%, respectively; while somewhat similar for *bla*$_{TEM}$ and *bla*$_{SHV}$, the rate of *bla*$_{CTX-M}$ was significantly higher in the present study. Additionally, Asnakew Abebe et al[8] reported lower values for *bla*$_{SHV}$ and *bla*$_{TEM}$, while their *bla*$_{CTX-M}$ rate was similar to the present study; however, the MDR rate (94%) in that study was higher. The findings of Arijit et al[20] also showed that 79.45% of the isolates were ESBL-positive and 87.6% were MDR, which are higher than those in the present study, although *bla*$_{TEM}$ played a significant role in both studies. Furthermore, despite differences in ESBL prevalence, the results reported by Pishtiwan,[21] Eftekhar,[22] and Aila[23] generally confirm the predominant role of bla genes and resistance to beta-lactams. Regarding the antibiotic resistance pattern, Abdullah et al.[24] reported high resistance to ampicillin, cefotaxime, cefepime, gentamicin, and ciprofloxacin, which is comparable to the results of the present study, although resistance to imipenem was considerably higher in their study. Similarly, studies by Lak Hondori[25] and Urmi[26] reported higher resistance to carbapenems than the present study, which may reflect differences in antibiotic selective pressure. Conversely, the study by Pishtiwan[21] demonstrated complete susceptibility to carbapenems, contrasting with the limited resistance (6%) observed in the present study. In the study by Gheitani,[27] resistance levels to most antibiotics were higher than in the present study, whereas the frequencies of the *bla*$_{IMP}$ and *bla*$_{VIM}$ genes were reported to be lower. In line with these findings, Beshah et al.[28] reported the presence of carbapenemase genes *bla*$_{IMP}$ and *bla*$_{VIM}$ in 16.7% and 0% of *K. pneumoniae* isolates, respectively, whereas in the present study these genes were detected in 22% and 26% of isolates, indicating a higher prevalence of metallo-beta-lactamase genes, particularly *bla*$_{VIM}$, among our isolates. Similarly, Shahkolahi et al.[29] reported complete resistance to ampicillin and a significant frequency of beta-lactamase genes, which was consistent with our findings, although the clonal diversity was greater in the present study. Regarding molecular typing, the identification of 31 pulsotypes in the present study demonstrates high genetic diversity and the concurrent circulation of multiple clones within the ICU. Compared to studies such as Esmaeilnia[30] (with more limited clustering) and Abdelbary[31] (17 pulsotypes), this finding indicates greater clonal diversity in our cohort. Furthermore, the lack of correlation between resistotype and pulsotype in the Abdelbary study, combined with the high diversity observed in the present study, suggests that the dissemination of resistance is not necessarily dependent on a specific clone. In this regard, Ahanjan et al.[32] also confirmed the pivotal role of ESBLs by reporting a high prevalence of ESBL production and complete resistance to cefotaxime, which aligns with the results of the present study, although differences in susceptibility to gentamicin were observed. Ultimately, the variations in resistance

profiles and gene frequencies across different studies could stem from differences in sample sizes, our single-center design restricted to a single city, and the relatively small cohort size. Additionally, screening a limited number of resistance genes and a specific panel of antibiotics, combined with a strict focus on hospitalized patients, may limit the generalizability of these findings to broader clinical or community settings.

6. Conclusion

In conclusion, this study highlights a critical and alarming prevalence of MDR: 68%, XDR: 58%, and PDR: 49% *K. pneumoniae* clinical isolates at Valiasr Hospital in Zanjan, Iran. This severe phenotypic resistance is driven by a high burden of ESBL genes, predominantly *bla*$_{TEM}$ (46%), *bla*$_{SHV}$ (44%), and *bla*$_{CTX-M}$ (40%), which demonstrated statistically significant correlations with widespread resistance to cephalosporins, monobactams, and non-beta-lactam agents. Furthermore, the emerging detection of metallo-beta-lactamase genes, including *bla*$_{VIM}$ (26%) and *bla*$_{IMP}$ (22%), poses an imminent threat to carbapenem efficacy. While high resistance was observed against standard therapies like ampicillin (90%), imipenem remains the most reliable last-line defense with a 76% susceptibility rate. These findings underscore the urgent need for strict antimicrobial stewardship programs and routine molecular surveillance to prevent the horizontal dissemination of these high-risk clonal lineages within hospital ICU settings.

Research Highlights

What Is Already Known?

The emergence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant strains has created significant challenges in the field of infection control and achieving effective treatments in hospital settings.

What Does This Study Add?

- A high prevalence of ESBL genes linked to multidrug resistance patterns in *K. pneumoniae* isolates in ICUs.
- The presence of carbapenemase genes (*bla*$_{IMP}$ and *bla*$_{VIM}$) indicates a shift toward carbapenem resistance.

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Author Contributions

Concept/Design: ZJ, RS; Data acquisition: ZJ; Data analysis and interpretation: HZ, LS; Drafting manuscript: ZJ, HZ, LS; Critical revision of manuscript: ZJ; Final approval and accountability: RS; Technical or material support: ZJ, RS; Supervision: RS.

Conflict of Interest Disclosures

All authors declared that they have no conflict of interest.

Ethical Approval

This study was approved by the Islamic Azad University, Zanjan Branch (Code: IR.IAU.Z.REC.1404.063).

References

1. Jafari Sales A, Jafari B, Beygoli N. Antimicrobial Resistance Patterns in Extended-spectrum betalactamase Producing *Klebsiella pneumoniae* Isolates in a Razi Hospital Marand, Iran. Electron J Biol. 2015;11(1):8-12. doi:10.13140/RG.2.2.36286.02883
2. Yamchlou N, Cinisli KT, Shadi-Dizaji A, Ghahremani Z, Golestani A, Soleymanpour K, et al. Investigating the antibacterial effects of Zataria multiflora methanolic extract on standard pathogenic bacteria in laboratory conditions. Int J Nat Life Sci. 2024;8(2): 102-10.
3. Abbas S, Kanwar R, Ullah K, Kanwal R, Tajamal M, Aslam MA, et al. Bacteriophage therapy: a possible alternative therapy against antibiotic-resistant strains of *Klebsiella pneumoniae*. Front Microbiol. 2025;16: 1443430. doi:10.3389/fmicb.2025.1443430
4. Jafari-Sales A, Soleimani H, Moradi L. Antibiotic resistance pattern in *Klebsiella pneumoniae* strains isolated from children with urinary tract infections from Tabriz hospitals. Heath Biotechnol Biopharma. 2020;4(1):38-45. doi:10.22034/HBB.2020.03
5. Hou H, Zhou X. Molecular mechanisms of *Klebsiella pneumoniae* pathogenicity: from virulence factors to emerging therapeutic strategies. Crit Rev Microbiol. 2026:1-32. doi:10.1080/1040841X.2026.2648208
6. Ding W, Wen Y, Chen L, Du H. Bacteriophage Therapy Against *Klebsiella Pneumoniae*. Microorganisms. 2026; 14(1):201. doi:10.3390/microorganisms14010201
7. Usman NI, Abdulwahab NM, Sulaiman MJ, Abdullahi SA. Multidrug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR) *Klebsiella pneumoniae* from clinical samples. SLU J Sci Technol. 2022;3:42-50.
8. Abebe AA, Birhanu AG, Tessema TS. Molecular characterization of virulence and antibiotic resistance genes in *Klebsiella pneumoniae* isolated from sputum samples at a tertiary hospital in Ethiopia. Sci Rep. 2026;16(1):8541. doi:10.1038/s41598-026-39069-3
9. Jafari-Sales A, Ghahremani Z, Golestani A, Ghorbanpour Koulani Jadid M, Hosseini-Karkaj K, Soleymanpour K, et al. Antibiotic resistance and blaTEM β-lactamase gene carriage in *Pseudomonas aeruginosa* isolates from burn patients: A multicenter study in Tabriz, 2024. Jorjani Biomed J. 2025;13(1):15-8. doi:10.29252/jorjanibiomedj.13.1.15
10. Jafari Sales A, Mobaiyen H, Farshbafi Nezhad Zoghi J, Nezamdoost Shadbad N, Purabdollah Kaleybar V. Antimicrobial resistance pattern of extended-spectrum β-Lactamases (ESBLs) producing *Escherichia coli* isolated from clinical samples in Tabriz city, Iran. Adv Environ Biol. 2014;8(16):179-82.
11. Jafari-Sales A, Golestani A, Ghahremani Z, Salek-Faramarzi M, Pashazadeh M, Dezfuli AA. Targeted mechanisms and novel therapeutic strategies against extended-spectrum beta-lactamases: From precise detection to intelligent management of bacterial resistance. J Curr Biomed Rep. 2025:51-7. doi:10.61882/jcbior.6.3.312
12. Jafari-Sales A, Shadi-Dizaji A. Molecular analysis of CTX-M genes among ESBL producing in *Pseudomonas aeruginosa* isolated from clinical samples by Multiplex-PCR. Hozan J Environment Sci. 2018;2(5): 17-29.

13. Zdarska V, Arcari G, Kolar M, Mlynarcik P. Antibiotic Resistance in *Klebsiella pneumoniae* and Related Enterobacterales: Molecular Mechanisms, Mobile Elements, and Therapeutic Challenges. Antibiotics. 2026;15(1):37. doi:10.3390/antibiotics15010037
14. Alishvandi A, Barancheshemeh M, Firuzpour F, Aram C, Kamali MJ, Keikha M. Decoding virulence and resistance in *Klebsiella pneumoniae*: pharmacological insights, immunological dynamics, and *in silico* therapeutic strategies. Microb Pathog. 2025;205: 107691. doi:10.1016/j.micpath.2025.107691
15. Husna A, Rahman MM, Badruzzaman AT, Sikder MH, Islam MR, Rahman MT, et al. Extended-spectrum β-lactamases (ESBL): challenges and opportunities. Biomedicines. 2023;11(11):2937. doi:10.3390/biomedicines11112937
16. Michaelis C, Grohmann E. Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics. 2023;12(2):328. doi:10.3390/antibiotics12020328
17. Nogueira LF, Maia MS, Clementino MA, Lima IF, Rodrigues JL, Fragoso LV, et al. Insights of β-Lactams Resistance in *Klebsiella pneumoniae* Clinical Isolates: A Focus on Molecular Identification of Drug Resistance. Int J Microb. 2026;2026(1):2707907. doi:10.1155/ijm/2707907
18. Maleki N, Tahanasab Z, Mobasherizadeh S, Rezaei A, Faghri J. Prevalence of CTX-M and TEM β-lactamases in *Klebsiella pneumoniae* isolates from patients with urinary tract infection, Al-Zahra hospital, Isfahan, Iran. Adv Biomed Res. 2018;7(1):10.
19. Mansagna CA, Baguy OM, Mariam K, Ibourahema C, Bertin TK, Bertin KG, et al. Prevalence of Beta-lactam Resistance Genes (Bla Genes) in Multidrug-Resistant Strains of *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Infections at a University Hospital in Abidjan, Ivory Coast. Microbiol Res J Int. 2026;36(6): 95-104.
20. Arijit Bora AB, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Giasuddin Ahmed GA. Prevalence of bla TEM, bla SHV and bla CTX-M genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian J Pathol Microbiol. 2014;57(2).
21. Pishtiwan AH, Khadija KM. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing *Klebsiella pneumoniae* and Escherichia coli isolated from thalassemia patients in Erbil, Iraq. Mediterr J Hematol Infect Dis. 2019;11(1):e2019041. doi:10.4084/MJHID.2019.041
22. Eftekhar F, Rastegar M, Golalipoor M, Mansoursamaei N. Detection of Extended Spectrum B-Lactamases in Urinary Isolates of *Klebsiella pneumoniae* in Relation to blaSHV, blaTEM and blaCTX-M gene carriage. Iran J Public Health. 2012;41(3):127-32.
23. El Aila NA, Ayesh NA. Prevalence of extended spectrum beta lactamase and molecular detection of blaTEM, blaSHV and blaCTX-M genotypes among Gram negative bacilli isolates from pediatric patient population in Gaza strip. BMC Infect Dis. 2023;23:1. doi:10.1186/s12879-023-08017-1
24. Abdullah AS, Hamad PA. Prevalence and molecular characterization of blaTEM, blaSHV, and blaCTX-M genes in ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from intensive care unit patients. Sci J Univ Zakho. 2026;14(1):104-11. doi:10.25271/sjuoz.2026.14.1.1627
25. Lak Hondori M, Mohammadi-Sichani M, Ghiasian M. Frequency of Beta-lactamase Genes (blaSHV, blaTEM, blaCTX-M-15, blaKPC, blaOXA-48) in Multidrug-resistant *Klebsiella pneumoniae*. Int J Mol Clin Microbiol. 2025;15(1):2180-9. doi:10.22034/ijmcm.2025.710258
26. Urmi UL, Nahar S, Rana M, Sultana F, Jahan N, Hossain B, et al. Genotypic to phenotypic resistance discrepancies identified involving β-lactamase genes, blaKPC, blaIMP, blaNDM-1, and blaVIM in uropathogenic *Klebsiella pneumoniae*. Infect Drug Resist. 2020:2863-75. doi:10.2147/IDR.S262493
27. Gheitani L, Fazeli H. Prevalence of blaVIM, blaIMP, and blaKPC genes among carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolated from Kurdistan and Isfahan hospitals, Iran. Res Mol Med. 2018;6(2): 12–20
28. Beshah D, Tessema TS, Woldemichael GB, Belachew EB, Alemayehu DH, Tesfaye A, et al. Resistance gene profiling of beta-lactamase and carbapenemase in gram-negative blood isolates: A tertiary care hospital. Plos One. 2026;21(3):e0344856. doi:10.1371/journal.pone.0344856
29. Shahkolahi S, Shakibnia P, Shahbazi S, Sabzi S, Badmasti F, Asadi Karam MR, et al. Detection of ESBL and AmpC producing *Klebsiella pneumoniae* ST11 and ST147 from urinary tract infections in Iran. Acta Microbiol IMM H. 2022;69(4):303-13. doi:10.1556/030.2022.01808
30. Esmaeilnia M, Saffari M, Rashki S, Marzhoseyni Z, Khaledi A, Moosavi GA, et al. Molecular typing of clinical and environmental isolates of *Klebsiella pneumoniae* producing ESBLs by PFGE. Iran J Basic Med Sci. 2022;25(2):208-13. doi:10.22038/IJBMS.2022.58445.12981
31. Mohamed ER, Aly SA, Halby HM, Ahmed SH, Zakaria AM, El-Asheer OM. Epidemiological typing of multidrug-resistant Klebsiella pneumoniae, which causes paediatric ventilator-associated pneumonia in Egypt. J Med Microbiol. 2017;66(5):628-34. doi:10.1099/jmm.0.000473
32. Ahanjan M, Naderi F, Solimanii A. Prevalence of Beta-lactamases genes and antibiotic resistance pattern of *Klebsiella pneumoniae* isolated from teaching hospitals, Sari, Iran, 2014. J Mazandaran Univ Med Sci. 2017;27(149):79-87.