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ORIGINAL ARTICLE

High prevalence of antimicrobial resistance genes in multidrug-resistant-ESBLs-producing *Klebsiella pneumoniae* post-COVID-19 pandemic

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ABSTRACT

Background and Objectives: *Klebsiella pneumoniae* is a common pathogen associated with healthcare-related infections. It is particularly notable for its ability to develop resistance to multiple antibiotics, making treatment challenging. During the COVID-19 pandemic, increased antibiotic use to manage critically ill patients was contributed to the rise of multidrug-resistant *Klebsiella pneumoniae*. This study evaluated the antibiotic resistance patterns of multidrug-resistant, ESBL-producing *Klebsiella pneumoniae* in northern Iran after the COVID-19 pandemic.

Materials and Methods: This cross-sectional study was conducted between September 2022 and October 2023. Clinical samples were collected from patients with nosocomial infections at hospitals in Sari. This study included 114 multidrug-resistant ESBLs-producing *Klebsiella pneumoniae* isolates. Antimicrobial susceptibility was assessed using broth macro-dilution, and resistance genes were detected by multiplex PCR.

Results: Gentamicin, ampicillin-sulbactam, co-amoxiclav, and ceftazidime displayed the lowest activity against multidrug-resistant *Klebsiella pneumoniae*. In contrast, piperacillin-tazobactam showed the highest activity. The prevalence of resistance genes was as follows: bla_{TEM} (99.12%), bla_{SHV} (74.56%), bla_{CTX} (88.60%), bla_{IMP} (64.04%), *acrA -B* (92.98%), and *OqXA -B* (67.54%).

Conclusion: This study identified over 50% of antibiotic-resistance genes. Over half of multidrug-resistant *Klebsiella pneumoniae* isolates showed resistance to antibiotics except piperacillin-tazobactam, which is recommended for treating multidrug-resistant *Klebsiella pneumoniae* infections.

Keywords: *Klebsiella pneumoniae*; Healthcare associated infections; COVID-19; Extended spectrum beta lactamase; Multidrug resistant; Multiplex polymerase chain reaction

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INTRODUCTION

The COVID-19 pandemic has significantly affected public health and healthcare, particularly in terms of antimicrobial resistance (AMR). Mortality rates for COVID-19 patients in intensive care units (ICUs) have increased notably. The rise of AMR has increased hospitalization duration and facilitated the emergence of multidrug-resistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* (MDR-ESBL *K. pneumoniae*). These strains can lead to various infections, including ventilator-associated pneumonia (VAP), urinary tract infections, and wound infections (1-3). The limited effectiveness of treatments for MDR-ESBL *K. pneumoniae* has made it a critically important pathogen (4).

Hypervigilance strains of *K. pneumoniae* can severely infect healthy individuals, while classic strains mainly affect hospitalized patients and produce Extended Spectrum Beta-Lactamases (ESBLs), leading to hospital-acquired infections (HAIs). The HAIs occur in hospitalized patients being treated for another condition. These infections can lead to serious health complications and may develop 48 to 72 hours after admission, within three days of discharge, or up to 30 days after surgery (5-8).

This bacterium uses various mechanisms to resist antibiotics, such as producing destructive enzymes, employing efflux pumps, losing porins, and altering target sites. *K. pneumoniae* carries antibiotic-resistance genes from both chromosomes and plasmids. The spread of these genes through plasmids and mutations has led to highly resistant strains of multidrug-resistant (MDR) *K. pneumoniae* in clinical settings, a growing concern over the past two decades. MDR strains show resistance to at least one agent in three or more antimicrobial classes (5, 6).

Prompt and effective treatment of infections caused by *K. pneumoniae* is crucial. The evaluation o*f K. pneumoniae* resistance mechanisms and epidemiology in hospital settings worldwide is essential. Despite critical literature reviews from various sources, the antibiotic resistance profile of *K. pneumoniae* isolates in the north of Iran is unclear after the COVID-19 pandemic. Resistance patterns and the prevalence of these genes vary globally across healthcare settings (6). Therefore, analyzing the resistance gene profiles in various regions is essential for understanding their distribution and can help develop effective control and treatment strategies against *K. pneumoniae*.

The high frequency of AMR in hospital settings poses a significant concern for both developed and developing countries. It has serious health implications as well as economic impacts. Clinical findings show that AMR leads to increased hospital infection rates and decreases the effectiveness of antibiotics. Consequently, this results in higher mortality rates, increased treatment costs, and longer hospital stays. It is essential to implement the precautions to prevent the spread of AMR in hospitals and develop effective treatment protocols for patients affected by these infections (5, 6).

Investigating the frequency of AMR genes in hospitals, particularly among patients with HAIs, is crucial in the post-COVID-19 pandemic era. Therefore, this study aimed to assess the frequency of genes associated with antibiotic resistance in multidrug-resistant extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* isolates from patients with HAIs at hospitals in northern Iran after the COVID-19 pandemic.

MATERIALS AND METHODS

Study design, and sample collection. This cross-sectional study was carried out at four teaching hospitals in Sari, Iran, between September 2022 and October 2023. The protocol received approval from the Ethics Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1401.158). Demographic data was collected from the patients' files (Table 1). Samples were collected from hospitalized patients with HAIs who had developed new infections after 72 hours, following CDC/NHSN surveillance definitions and criteria for specific types of HAIs (Table 1) (7). This study specifically focused on MDR-ESBL *K. pneumoniae* isolates. Outpatients, Gram-positive isolates, and any *K. pneumoniae* isolates that were not resistant to at least one agent in three or more antimicrobial classes were excluded from the study. To identify, the samples were sent to the central microbiology laboratory. As part of the routine procedure, all samples were cultured on MacConkey and Blood agar (QUELAB, USA) plates. Standard microbiological procedures were followed to identify *K. pneumoniae* (8-10).

Antimicrobial susceptibility assays and phenotypic screening of ESBLs-producing strains.

Table 1. The demographic of patients with HAIs caused by MDR-ESBL *K. pneumoniae*.

Variable	N (%)
Age	
< 1 Years old	4 (3.51)
1-18 Years old	16 (14.04)
> 18 Years old	94 (82.46)
Gender	
Male	67 (58.77)
Female	47 (41.23)
Unit	
ICU	41 (35.96)
CCU	15 (13.16)
Emergency	12 (10.53)
PICU	9 (7.89)
Internal	8 (7.02)
Neurology	7 (6.14)
Surgery	6 (5.26)
NICU	4 (3.51)
Orthopedic	3 (2.63)
OR	3 (2.63)
Outpatient	2 (1.75)
BICU	1 (0.88)
Oncology	1 (0.88)
ENT	1 (0.88)
Psychiatric	1 (0.88)
Sample	
UTI	44 (38.60)
Sputum	38 (33.33)
Blood	14 (12.28)
Wound	8 (7.02)
Catheter	5 (4.39)
Abscess	4 (3.5)
CSF	1 (0.88)

The susceptibility of the *K. pneumoniae* isolates to commonly used antibiotics was assessed using the standard broth macro-dilution technique based on the Clinical & Laboratory Standards Institute (CLSI 2020) (10-12). To prepare the macro dilution series, reagent powders of antibiotics including; ampicillin-sulbactam, ceftazidime, cefepime, ciprofloxacin, colistin, co-amoxiclav, gentamicin, meropenem, and piperacillin-tazobactam were obtained from their respective companies (Bio basic, Canada). Strains that were resistant to at least one agent in three or more antimicrobial classes were described as MDR isolates.

To identify ESBL-producing *K. pneumoniae* isolates, a combined disk test (CDT) was employed. The following cephalosporins were used: cefotaxime, cefepime, ceftazidime, and ceftriaxone (Padtan Teb, Iran) to identify ESBL-producing strains. Additionally, the presence of ESBLs using ceftazidime/clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime/clavulanic acid (30/10 µg), and cefepime/clavulanic acid (30/10 µg) antibiotic disks (Padtan Teb, Iran) were detected. A standard strain of *K. pneumoniae*, ATCC No.700603, was used as a positive control in this assay (8-12).

Detection of antibiotic-resistant genes in MDR-ESBLs *K. pneumoniae* isolates. According to the company's guidelines, bacterial DNA was extracted using a commercially available DNA extraction kit (Yekta Tajhiz, Iran). The Multiplex PCR method was used to screen all isolates for ESBLs genes (bla_{TEM}, bla_{CTX}, and bla_{SHV}), MBLs genes (bla_{IMP}), as well as efflux pumps (*acr AB* and *oqx AB*). The specific primers were utilized for this purpose. In summary, the amplification process involved a denaturation step at 94°C for 30 seconds, followed by 35 cycles at 64°C, 72°C for 30 minutes, and a final extension step at 72°C for 10 minutes. The positive control strains used in this study included *K. pneumoniae* ATCC NO.51503 (bla_{CTX}), and *K. pneumoniae* ATCC NO. 700603 (bla_{SHV}), and *E. coli* NCTC NO. 13476 (bla_{IMP}) (8-20).

Statistical analysis. Data were analyzed using SPSS version 22. Statistical analysis involved Chi-square and Fisher's exact tests.

RESULTS

In current study, a total of 12834 clinical samples were collected from patients with HAIs at four hospitals in Sari, which were designated as coronavirus centers. Out of these samples, 205 isolates were identified as *K. pneumonia*, with 114 of these isolates classified specifically as MDR-ESBL *K. pneumoniae* isolates. The median age of the patients was 58.50 years, with an interquartile range (IQR) of 34.75 to 72. The majority of the patients were male (58.77%). Among the patients, 35.96% were hospitalized in the ICU. The most frequently collected samples from patients with HAIs were from urinary tract infection (UTI) (38.60%) and sputum (33.33%) (Table 1).

Table 2, summarizes the antibiotic susceptibilities,

Table 2. Susceptibilities to antibiotics in MDR -ESBL *K. pneumoniae* isolates

Antibiotic agents	Resistant	Intermediate	Sensitive	MIC$_{50}$	MIC$_{90}$	GM MIC	Mode
Aminoglycosides							
Gentamicin	73.68%	7.89%	18.42%	500	1000	145.77	1000
Beta-lactam/Beta-lactamase inhibitor							
Ampicillin Sulbactam	80.70%	3.51%	15.79%	500	1000	181.53	1000
Co-amoxiclav	100%	0	0	500	1000	487.99	1000
Carbapenems							
Meropenem	62.28%	5.26%	32.46%	7.8	250	10.11	0.9
Cephalosporins							
Ceftazidime	84.21%	1.75%	14.04%	500	1000	169.29	1000
Cefepime	80.70%	6.14%	13.16%	187.5	1000	87.63	500
Fluoroquinolones							
Ciprofloxacin	80.70%	9.65%	9.65%	125	500	53.11	250
Penicillins/ß-lactamase inhibitors							
Piperacillin-Tazobactam	41.23%	14.91%	43.86%	62.5	500	46.84	1.9
Polymyxins							
Colistin	85.96%	0	14.04%	7.8	1000	23.86	3.9

MIC$_{50}$, the concentration at which 50% of the isolates were inhibited; MIC$_{90}$, the concentration at which 90% of the isolates were inhibited; GM, geometric mean

including the MIC$_{50}$, MIC$_{90}$, MIC GM, and mode of MICs for ampicillin-sulbactam, ceftazidime, cefepime, ciprofloxacin, colistin, co-amoxiclav, gentamicin, meropenem, and piperacillin-tazobactam against MDR-ESBL *K. pneumoniae*. These strains exhibited the lowest susceptibility rates to gentamicin, ampicillin-sulbactam, co-amoxiclav, ceftazidime, and meropenem. On the other hand, MDR-ESBL *K. pneumoniae* showed the highest susceptibility rates to piperacillin-tazobactam. The frequency of resistance genes was *bla*$_{SHV}$ (74.56%), *bla*$_{CTX}$ (88.60%), *bla*$_{TEM}$ (99.12%), *acrAB* (92.98%), *OqxAB* (67.54%), and *bla*$_{IMP}$ (64.04%) respectively. The *KPC* was not detected (Table 3 and Fig. 1). Table 4, illustrates the significant correlation between antibiotic sensitivity in MDR-ESBL *K. pneumoniae* isolates and the presence of antibiotic-resistance genes.

Also, our analysis identified fourteen genotypes among the isolates, all of which contained at least four antibiotic-resistance genes. The most common genotypes were co-presence of (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$, *acrA, acrB, oqXB, oqXA,* and *bla*$_{IMP}$) in 32 (30.77%) isolates, (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$, *acrA, acrB, oqXB,* and *oqXA*) in 18 (17.31%) isolates, (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$, *acrA, acrB, oqXB,* and *bla*$_{IMP}$) in 15 (14.42%) isolates, (*bla*$_{TEM}$, *bla*$_{CTX}$, *acrA, acrB, oqXB, oqXA,* and *bla*$_{IMP}$), (*bla*$_{TEM}$, *bla*$_{CTX}$, *acrA, acrB, oqXB,* and *oqXA*), (*bla*$_{TEM}$, *bla*$_{CTX}$, *acrA, acrB, oqXB,* and *bla*$_{IMP}$) in 6 (5.77%) isolates, and (*bla*$_{TEM}$, *bla*$_{SHV}$, *acrA, acrB, oqXB, oqXA,* and *bla*$_{IMP}$) in four (3.85%) isolates, respectively.

Table 3. Distribution of antibiotic resistance genes in MDR -ESBL *K. pneumoniae* isolates

Genes (Positive)	N (%)
bla$_{TEM}$	113 (99.12)
bla$_{SHV}$	85 (74.56)
bla$_{CTX}$	101 (88.60)
bla$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX}$	75 (65.79)
bla$_{IMP}$	73 (64.04)
acrA, acrB	106 (92.98)
OqXB, oqXA	77 (67.54)

DISCUSSION

The current study revealed that MDR-ESBL *K. pneumoniae* was responsible for 43.86% of HAIs in northern Iran. Most of the MDR-ESBL *K. pneumoniae* strains were isolated from UTI samples in patients admitted to the ICU. The macro dilution method showed that these strains had the lowest suscep-

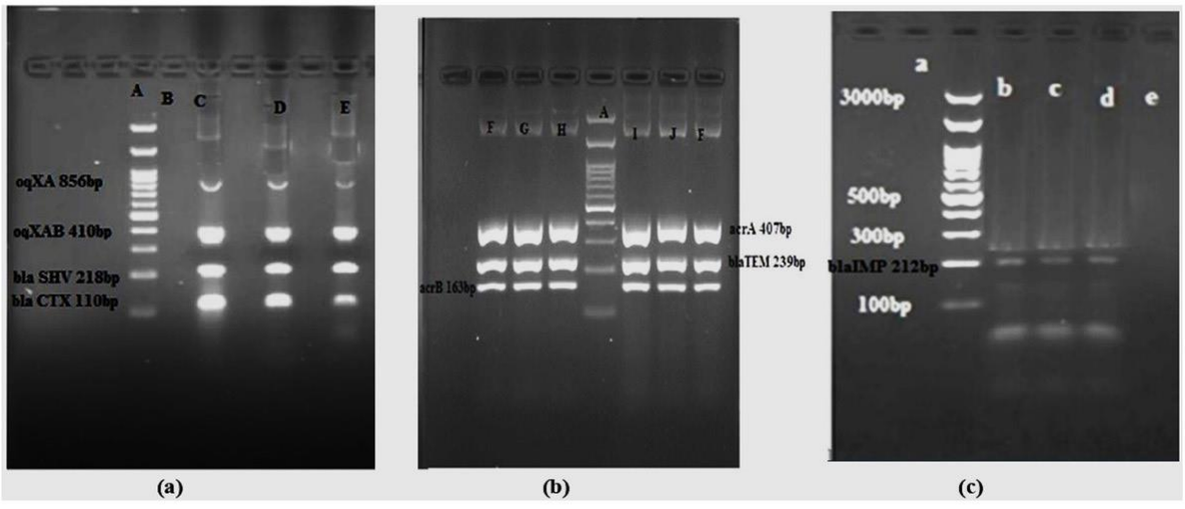

Fig. 1. Gel electrophoresis of the PCR products of antibiotic resistance genes in *K. pneumoniae* isolates.
(a): (A): ladder 100-3000 bp; (B): negative control; (C): positive sample for genes of bla_{SHV} (218 bp), bla_{CTX} (110 bp), *OqXB* (410 bp), *OqXA* (856 pb); (D, E): samples.
(b): (A): ladder 100-3000 bp; (F): positive sample for genes of *acrA* (407 bp); *acrB* (163 bp); bla_{TEM} (239 bp); (G, H, I, J): samples.
(c): (a): ladder 100-3000 bp; (b): positive sample for gene of bla_{IMP} (212 bp); (c, d): samples; (e): negative control.

Table 4. Susceptibilities to antibiotics in MDR -ESBL *K. pneumoniae* isolates

Positive Genes	Antibiotic	MIC Result			P- value
		Resistant	Intermediate	Sensitive	
bla_{TEM}	Gentamicin	73.45%	18.58%	7.96%	1.000
	Colistin	85.84%	14.16%	0	1.000
	Ciprofloxacin	80.53%	9.73%	9.73%	1.000
	Ceftazidime	84.07%	14.16%	1.77%	1.000
	Meropenem	62.83%	32.74%	4.42%	0.053
	Ampicillin Sulbactam	81.42%	15.04%	3.54%	0.193
	Piperacillin-Tazobactam	41.59%	44.25%	14.16%	0.149
	Cefepime	81.42%	13.27%	5.31%	0.061
	Co-amoxiclav	100%	0	0	NA
bla_{SHV}	Gentamicin	81.18%	11.76%	7.06%	0.004
	Colistin	84.71%	15.29%	0	0.508
	Ciprofloxacin	82.35%	7.06%	10.59%	0.284
	Ceftazidime	82.35%	15.29%	2.35%	0.866
	Meropenem	63.53%	29.41%	7.06%	0.250
	Ampicillin Sulbactam	80%	15.29%	4.71%	0.738
	Piperacillin-Tazobactam	45.88%	38.82%	15.29%	0.156
	Cefepime	81.18%	12.94%	5.88%	1.000
	Co-amoxiclav	100%	0	0	NA
bla_{CTX}	Gentamicin	77.23%	16.83%	5.94%	0.024
	Colistin	87.13%	12.87%	0	0.319
	Ciprofloxacin	79.21%	10.89%	9.90%	0.649
	Ceftazidime	85.15%	12.87%	1.98%	0.525
	Meropenem	61.39%	33.66%	4.95%	0.519
	Ampicillin Sulbactam	82.18%	13.86%	3.96%	0.267

Table 4. Continuing ...

	Piperacillin-Tazobactam	43.56%	41.58%	14.85%	0.371
	Cefepime	80.20%	12.87%	6.93%	1.000
	Co-amoxiclav	100%	0	0	NA
bla_{IMP}	Gentamicin	78.08%	15.07%	6.85%	0.358
	Colistin	86.30%	13.70%	0	0.890
	Ciprofloxacin	90.41%	2.74%	6.85%	0.001
	Ceftazidime	94.52%	5.48%	0	<0.001
	Meropenem	97.26%	0	2.74%	<0.001
	Ampicillin Sulbactam	83.56%	12.33%	4.11%	0.495
	Piperacillin-Tazobactam	49.32%	31.51%	19.18%	0.002
	Cefepime	90.41%	9.59%	0	<0.001
	Co-amoxiclav	100%	0	0	NA
acrA	Gentamicin	73.45%	18.58%	7.96%	1.000
	Colistin	85.84%	14.16%	0	1.000
	Ciprofloxacin	80.53%	9.73%	9.73%	1.000
	Ceftazidime	84.07%	14.16%	1.77%	1.000
	Meropenem	62.83%	31.86%	5.31%	0.377
	Ampicillin Sulbactam	80.53%	15.93%	3.54%	1.000
	Piperacillin-Tazobactam	40.71%	44.25%	15.04%	0.561
	Cefepime	80.53%	13.27%	6.19%	1.000
	Co-amoxiclav	100%	0	0	NA
acrB	Gentamicin	73.58%	19.81%	6.60%	0.114
	Colistin	84.91%	15.09%	0	0.236
	Ciprofloxacin	82.08%	9.43%	8.49%	0.181
	Ceftazidime	83.96%	14.15%	1.89%	1.000
	Meropenem	62.26%	33.02%	4.72%	0.526
	Ampicillin Sulbactam	80.19%	16.04%	3.77%	1.000
	Piperacillin-Tazobactam	42.45%	41.51%	16.04%	0.262
	Cefepime	81.13%	12.26%	6.60%	0.592
	Co-amoxiclav	100%	0	0	NA
oqXB	Gentamicin	75.93%	17.59%	6.48%	0.028
	Colistin	87.04%	12.96%	0	0.162
	Ciprofloxacin	83.33%	6.48%	10.19%	0.002
	Ceftazidime	84.26%	13.89%	1.85%	1.000
	Meropenem	63.89%	30.56%	5.56%	0.244
	Ampicillin Sulbactam	80.56%	15.74%	3.70%	1.000
	Piperacillin-Tazobactam	43.52%	40.74%	15.74%	0.024
	Cefepime	81.48%	12.96%	5.56%	0.327
	Co-amoxiclav	100%	0	0	NA
oqXA	Gentamicin	74.36%	19.23%	6.41%	0.673
	Colistin	85.90%	14.10%	0	1.000
	Ciprofloxacin	84.62%	6.41%	8.97%	0.203
	Ceftazidime	80.77%	16.67%	2.56%	0.331
	Meropenem	58.97%	34.62%	6.41%	0.619
	Ampicillin Sulbactam	76.92%	19.23%	3.85%	0.325
	Piperacillin-Tazobactam	41.03%	44.87%	14.10%	0.962
	Cefepime	78.21%	15.38%	6.41%	0.635
	Co-amoxiclav	100%	0	0	NA

R, Resistant; S, sensitive; I, intermediate; significant

P value is shown in boldface

tibility rates to gentamicin, ampicillin-sulbactam, co-amoxiclav, ceftazidime, and meropenem while demonstrating the highest susceptibility rates to piperacillin-tazobactam. MDR-ESBL *K. pneumoniae* is a challenging nosocomial pathogen linked to high mortality rates, prolonged hospitalization duration, and increased healthcare costs worldwide (17, 18).

Before the COVID-19 pandemic, our surveillance study in northern Iran found that the occurrence of nosocomial pneumonia caused by ESBL-producing isolates was notably low at 14.63% (19, 20). The inappropriate use of antibiotics at the onset of the COVID-19 pandemic may have contributed to the increase in AMR. During the COVID-19 pandemic, 70% of diagnosed patients were prescribed antibiotics, leading to increased risks of HAIs and AMR. This overuse has become a significant public health concern, especially with co-infections reported in some regions at rates between 0.35% and 53%. The effort to prevent secondary infections has further aggravated antibiotic resistance (21).

In 2014, a survey reported the most common HAIs as follows: 50.81% wound infections, 21.31% respiratory infections, 19.67% UTIs, and 8.19% blood infections (22). In contrast, our post-COVID-19 survey revealed a shift: UTIs (38.6%), respiratory infections (33.33%), and blood infections (12.8%) were the most prevalent in ICU. Notably, MDR-ESBL isolates accounted for 38.6% of UTIs, raising concerns due to their associations with severe complications like urinary catheter-related infections. Similar to our study, Miftode et al., identified *K. pneumonia* as a significant pathogen in UTIs (23). The increasing presence of MDR-ESBL *K. pneumonia* isolates in UTIs is alarming following the COVID-19 pandemic. Recent reports indicate that up to 80% of COVID-19 patients admitted to the ICU require invasive procedures, such as urinary catheters and mechanical ventilation. The combination of both invasive and non-invasive methods, along with prolonged ICU hospitalization and a high reliance on antibiotics, raises the risk of hospital-associated infections in this setting (21).

The current study revealed a significant level of resistance (ranging from 41.23% to 100%) to different classes of antibiotics. However, piperacillin-tazobactam was detected to be the most effective antibiotic against MDR -ESBL *K. pneumoniae* isolates. Also, Post-COVID-19, Rahimzadeh et al., reported even higher resistance rates: 91.23% to aminoglycosides, 96.49% to fluoroquinolones, 84.21% to colistin, 90.35% to ceftazidime, and 75.44% to carbapenems (21). But, in our studies conducted in northern Iran before the COVID-19 outbreak, we observed that 53.6% of Gram-negative isolates were resistant to aminoglycosides, 85.7% to ciprofloxacin, 35.7% to colistin, and 57.1% to imipenem. Rezai et al., reported that ESBL-producing isolates from patients with ventilator-associated pneumonia had resistance rates of 79% to aminoglycosides, 82.8% to fluoroquinolones, and 89.7% to cephalosporins (19, 20).

AMR in Gram-negative bacteria (GNB) has escalated during the COVID-19 era for several reasons. These include prior exposure to antibiotics, the use of broad-spectrum antibiotics for COVID-19 patients, challenges in differentiating between viral and bacterial infections, prolonged mechanical ventilation, changes in hospital operations, and transmission by healthcare workers (21, 24). It highlights the need for effective infection control measures and strategies to tackle antibiotic resistance.

Our results indicated that the most commonly detected ESBL gene was bla_{TEM}, found in 99.12% of the samples, followed by bla_{CTX} in 88.60% and bla_{SHV} in 74.56%. Furthermore, our study revealed a statistically significant relationship (P= 0.004, 0.024) between the occurrence of the bla_{SHV} and bla_{CTX} genes and resistance to aminoglycosides. A systematic review in Iran found that the bla_{TEM} gene is the most prevalent ESBL-producing gene, identified in 51% of cases (25). Supporting research shows bla_{TEM} at 49%, followed by bla_{SHV} at 44% and bla_{CTX} at 28% (26). A study by Bagheri-Nesami et al., reported even higher frequencies: 94.3% for bla_{SHV} and 48.6% for bla_{CTX} (20). A meta-analysis indicated a 14% prevalence of ESBL strains, with a 5.38% annual increase. In Asia and Africa, prevalence ranges from 15% to 46%, significantly higher than the 2% to 6% seen in Europe and the Americas. The findings indicate an excessive use of broad-spectrum antibiotics in healthcare settings, which contributes to the transmission of ESBL-encoding genes on plasmids. Some of these genes are located within transposons or integrons, enabling their transfer between different organisms. These resistant strains present a significant therapeutic challenge, as they often show resistance to various antimicrobial drugs, including aminoglycosides, quinolones, and cotrimoxazole (27, 28).

Our results indicated that 64.04% of the isolates contained the bla_{IMP} gene in isolates which revealed a statistically significant relationship (P <0.001) be-

tween the occurrence of this gene and resistance to Meropenem. Before the COVID-19 pandemic, our study demonstrated that MDR Gram-negative isolates exhibited the highest susceptibility to carbapenems (66%), and Bagheri-Nesami et al., noted that Enterobacteriaceae were generally sensitive to carbapenems (19, 20). Our results highlight a serious concern regarding the spread of carbapenem-resistant Gram-negative and underscore the need for further investigation to understand and address this issue. Another study assayed the causes of secondary infections in COVID-19 patients and found that *K. pneumoniae* was the most frequently identified bacterium, followed closely by *A. baumannii*. Notably, the researchers observed a 6% increase in resistance to carbapenems among the bacterial isolates (29). Additionally, during the pandemic, the prevalence of carbapenem-resistant Enterobacteriaceae in Italy rose dramatically from 5% in 2019 to 50% (30).

This study detected that *acrAB* was the most common gene in MDR-ESBL *K. pneumoniae* isolates, present in 92.98% of the samples. *OqxAB* was found in 67.54% of the isolates. Additionally, the *OqxB* gene and resistance to ciprofloxacin were statistically significant (P = 0.002). The *acrAB* and *OqxAB* genes encode efflux pumps that play a crucial role in removing antibiotics, such as cephalosporins, carbapenems, and fluoroquinolones, from the cell membrane of *K. pneumoniae*. Previous studies in various regions of Iran reported that the resistance of Gram-negative bacteria to ciprofloxacin ranged from 10.2% to 85% (31). Abdi et al. reported a slightly lower resistance rate of 51%. The resistance rate in the United States is 16.8%, while in China, it varied from 43% in 2013 to 48% in 2015 (32, 33). Factors contributing to this distinction include variations in sample sizes, types of HAIs, geographical locations, patient wards, and antibiotic prescription patterns.

In the present study, the ratio of resistance to colistin was 85.96%. Colistin remains highly effective against most isolates of *K. pneumoniae*; however, the decreasing effectiveness against carbapenem-resistant strains is concerning. The increased use of colistin has led to outbreaks involving species that are inherently resistant to polymyxins and has contributed to the rising number of colistin-resistant *K. pneumoniae* strains. Research shows a correlation between the treatment of infections caused by carbapenem-resistant *K. pneumoniae* with colistin and the emergence of colistin-resistant strains. For instance, a study by Mansour et al., in Tunisia found that out of 29 carbapenem-resistant *K. pneumoniae* isolates, 7 (24.1%) were resistant to colistin (34). A meta-analysis indicated a strong association between carbapenem-producing *K. pneumoniae* and increased resistance to colistin, revealing that 31.7% of *K. pneumoniae* isolates producing carbapenemase were resistant to colistin (35). Therefore, it is crucial to continually monitor the use of colistin. The current study had some limitations, notably the absence of sequencing for MDR and ESBL-producing genes, which could significantly enhance our understanding of the findings.

CONCLUSION

This study identified a high prevalence of antibiotic-resistant genes and an increase in multi-drug resistant extended-spectrum beta-lactamase (MDR-ESBL) *K. pneumoniae* isolates in patients with healthcare-associated infections (HAIs) in the intensive care unit (ICU) at north hospitals in Iran. It is recommended to prescribe piperacillin-tazobactam for the empirical treatment of infections caused by MDR-ESBL *K. pneumoniae* at hospitals in northern Iran.

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