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Research Article

Prevalence of *bla* $_{VIM}$, *bla* $_{IMP}$, and *bla* $_{KPC}$ Genes Among Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) Isolated from Kurdistan and Isfahan Hospitals, Iran

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Abstract

Background: Carbapenem resistance among *Klebsiella pneumoniae* is an emerging problem worldwide. One of the main mechanisms of resistance to carbapenems is the potential of *Klebsiella pneumoniae* to produce carbapenemase enzyme.

This study was conducted to determine the frequency of bla_{VIM}, bla_{IMP}, and bla_{KPC} among carbapenem-resistant *K. pneumoniae* (CRKP) isolated from Kurdistan and Isfahan hospitals.

Materials and Methods: This study was carried out in Iran using 183 samples from the Besat and Alzahra hospitals in 2017. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion. The modified Hodge test (MHT) was used to investigate the presence of carbapenemase. The β -lactamases genes were detected by PCR.

Results: The highest and lowest rates of resistance were observed against cefotaxime (98.2%) and gentamicin (43.6%), respectively. Among the 183 isolates, 134 (73.2 %) were positive by the MHT. The prevalence rates of bla_{VIM}, bla_{IMP}, and bla_{KPC} were 4 (2.18%), 1 (0.5%), and 0%, respectively.

Conclusion: The prevalence of CRKP strains is a major concern and infection control processes are needed. These gene showed a low prevalence in our country, likely because other mechanisms of resistance to carbapenems are involved.

Keywords: bla_{VIM}, bla_{IMP}, bla_{KPC}, Carbapenemase, *Klebsiella pneumoniae*

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1. Introduction

Klebsiella pneumoniae is a Gram-negative, facultative anaerobic bacterium that can cause different types of healthcare-associated infections, including pneumonia, blood-stream infections, wound or surgical site infections, and meningitis [1]. Antibiotic resistance has become a major problem worldwide. Antibiotic multi-drug resistant in *K. pneumoniae* is conferred primarily by extended spectrum β-lactamase (ESBL), which are enzymes that hydrolyze the β-lactam ring of β-lactam antibiotics [2]. Broad-spectrum antibiotics belonging to carbapenems are a group of antibiotics useful for treating

multi-drug resistant *K. pneumoniae* because of their stability against β-lactamase hydrolysis [3]. Carbapenems are the last-line therapy for nosocomial infections. They have a broad spectrum of activity and stability compared to most β-lactams. The bipolar structure of these antibiotics helps them to cross easily through outer membrane proteins in the gram-negative bacterial cell wall to target penicillin-binding proteins [4]. However, the emergence of carbapenem-resistant *K. pneumoniae* has become an increasingly serious public health problem [5]. There are 3 mechanisms of resistance to carbapenems, 1-producing carbapenemase enzymes, 2-porin loss, and 3-expression of efflux pumps, the first of which is the major threat [6]. Numerous carbapenemases have been reported, including KPC, GES, SME, NMC-A, and IMI types (Amber class A), IMP, VIM, and NDM types (Amber class B), metallo-β-lactamases and OXA types (Amber class D), and oxacillinases [7]. Because information on the carbapenemase enzyme is limited in Iran, identifying these pathogens is a major challenge for diagnostic laboratories. Thus, the aim of this study was to determine the frequency of bla_{VIM}, bla_{IMP} and bla_{KPC} among carbapenem-resistant *K. pneumoniae* (CRKP) isolated from hospitals in Kurdistan and Isfahan.

2. Materials and Methods

2.1. Patient and samples

This cross-sectional study was carried out using 183 samples from Besat and Alzahra hospitals in 2017. Any clinical specimens such as tracheal aspirate, blood, urine, urethral catheter, wound were examined to detect *K. pneumoniae*. All samples were cultured on a specific medium and colonies showing the characteristics of gram-negative bacteria were isolated. *Klebsiella pneumoniae* isolates were detected by IMVIC standard biochemical tests (all samples were citrate-positive, nonmotile, Voges-Proskauer-positive, methyl red-negative, and lactose fermenters).

2.2. Antibiotic susceptibility test

All antibiotic disks including ceftazidime (30 μg), imipenem (10 μg), meropenem (10 μg), co-trimoxazole (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), cefotaxime (30 μg), cefepime (30 μg), ampicillin (10 μg), amikacin (30 μg), tetracycline (10 μg), and cefoxitin (30 μg) were purchased from Mast Group (Merseyside, UK). An antibiogram assay was performed using Kirby-Bauer antimicrobial disk diffusion according to the CLSI standard on the Mueller Hinton Agar plates (Merck, Billerica, MA, USA) [8]. A standard isolate of *Escherichia coli* ATCC 25922 was used as a quality control strain in antimicrobial susceptibility testing [9, 10].

2.3. Modified Hodge test (MHT)

The Modified Hodge Test (MHT) was performed according to CLSI recommendations. AN aliquot of *E. coli* ATCC25922 in 5 mL saline was adjusted to 0.5 McFarland standard,

and then the suspension was diluted by 1:10. Next, a sterile cotton swab was dipped into the suspension and used to inoculate a Muller-Hinton agar plate, after which a 10-μg meropenem disk was placed in the center of the plate. Using a sterile swab, suspected bacteria (resistant or semi-susceptible isolates to one or more antibiotics in the carbapenem family and third-generation cephalosporins) were streaked in a straight line from the edge of the meropenem disc onto the plate edge. The plate was incubated overnight at 35 ± 2°C in ambient air for 16–24 h. In negative isolates, the clear zones around the disk remained homogeneous, while carbapenemase-producing isolates caused a cloverleaf-like indentation. The *K. pneumoniae* ATCC®BAA-1705^{TM} and *K. pneumoniae* ATCC®BAA-1706^{TM} (ATCC; Manassas, VA, USA) were used as positive and negative controls, respectively.

2.4. Molecular detection of *bla* $_{VIM}$, *bla* $_{IMP}$, and *bla* $_{KPC}$ genes by PCR

PCR was used to screen for *bla* $_{VIM}$, *bla* $_{IMP}$, and *bla* $_{KPC}$. The primers used to detect these genes were as follows: For bla_{VIM}: VIM-F (5′-GTGTTTGGTCGCATATCGC-3′) and VIM-R (5′-CGCAGCACCAGGATAGAAG-3′), for bla_{IMP}: IMP-F (5′-GGAATAGAGTGGCTTAATTC-3′) and IMP-R (5′-GCCAAGCTTCTATATTTGCG-3′), for bla_{KPC} : KPC-F (5′-TCTGGACCGCTGGGAGCTGG-3’) and KPC-R (5’- TGCCCGTTGACGCCCAATCCC-3’) [11, 12]. PCR was performed in separate reactions containing the DNA template, specific forward/reverse primers, and commercial master mix (Bioneer, Daejeon, Korea). Amplification was carried out under the following thermal cycling conditions:

initial denaturation at 95°C for 10 min, followed 36 cycles at 94°C for 1 min, annealing at 63°C for 1 min, extension at 72°C for 1 min; and final extension of 72°C for 5 min.

The final products of PCR were electrophoresed on an agarose gel [12].

3. Results

During the study period, a total of 183 cases of *K. pneumoniae* isolates were collected from different clinical samples at the studied hospitals. Overall, 106 (58%) *K. pneumoniae* isolates were obtained from female patients and 77 (42%) were from male patients, ranging from 2 to 87 years old. One hundred twenty-three (67.2%) isolates of *K. pneumoniae* were from intensive care units (ICUs), 24 (13.1%) were from internal medicine wards, 17 (9.3%) were from emergency wards, 13 (7.1%) were from surgery wards, and 6 (3.3%) were from infant wards. The most frequent infections associated with clinical isolates of *K. pneumoniae* were urinary tract infections (52.5%), followed by tracheal (21.3%), bronchial (8.7%), catheter (6.1%), abdominal fluid (4.9%), blood (3.8%), and cerebrospinal fluid (2.7%).

The profile of antibiotic susceptibility was determined by the disc diffusion method. As shown in Table 1, the highest and lowest rates of resistance were observed for cefotaxime (98.2%) and gentamicin (43.6%), respectively. The modified Hodge test was performed for suspected carbapenemase-producing isolates (Figure 1). A total of 73.2% (134 of 183) isolates were positive according to the MHT. Among MHT-positive isolates,

urine samples comprised most cases (69.5%), while cerebrospinal fluids showed the lowest rate of positivity (1.3%). Additionally, ICU wards with 95 (70.1%) and infant ward with 3 (2.2%) samples were the most and least frequent cases in the MHT-positive group, respectively.

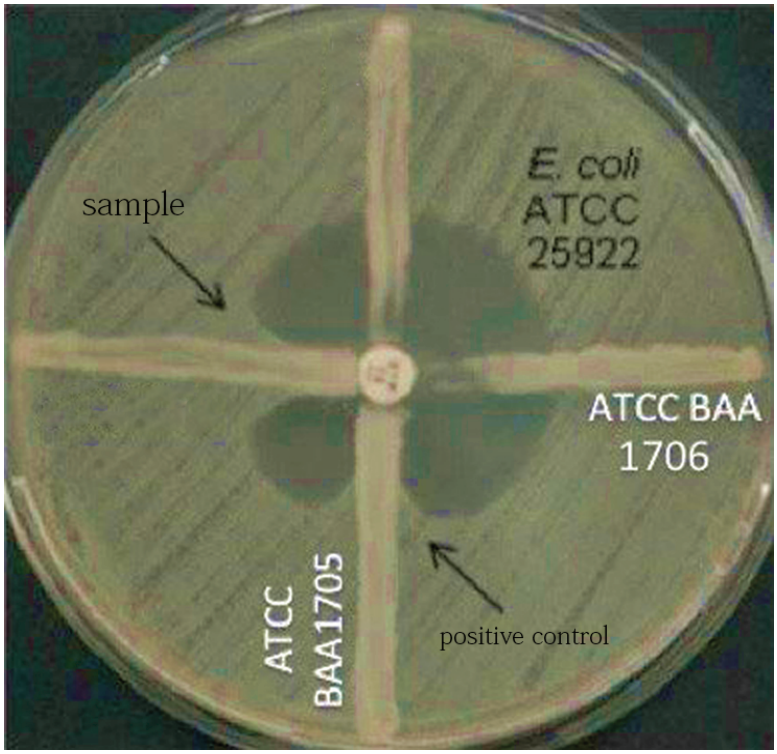

Figure 1: Clover leaf test or modified Hodge test (MHT).

Table 1: Antimicrobial resistance profile of *Klebsiella pneumoniae* isolates.

Antibiotics	**Resistant** (No. (%))	**Intermediate** (No. (%))	**Susceptible (**No. (%))
Gentamicin	80 (43.6%)	9 (5.2%)	94 (51.2%)
Ampicillin	172 (94.3%)	7 (3.6%)	4 (2.1%)
Amikacin	146 (79.8%)	12 (6.4%)	25 (13.8%)
Imipenem	129 (70.5%)	44 (24.3%)	10 (5.2%)
Ciprofloxacin	167 (91.5%)	4 (2%)	12 (6.5%)
Meropenem	137 (74.9%)	26 (14.2%)	20 (10.9%)
Ceftazidime	176 (96.1%)	0%	7 (3.9%)
Cefotaxime	180 (98.2%)	0%	3 (1.8%)
Cefoxitin	149 (81.5%)	25 (13.6%)	9 (4.9%)
Co-trimoxazole	154 (84.2%)	22 (12.1%)	7 (3.7%)
Tetracycline	157 (85.9%)	3 (1.5%)	23 (12.6%)
Cefepime	166 (90.6%)	11 (6%)	6 (3.4%)

The molecular assay of β-lactamases genes revealed that the prevalence of bla_{VIM}, bla_{IMP}, and bla_{KPC} was 4 (2.18%), 1 (0.5%), and 0%, respectively (Figure 2).

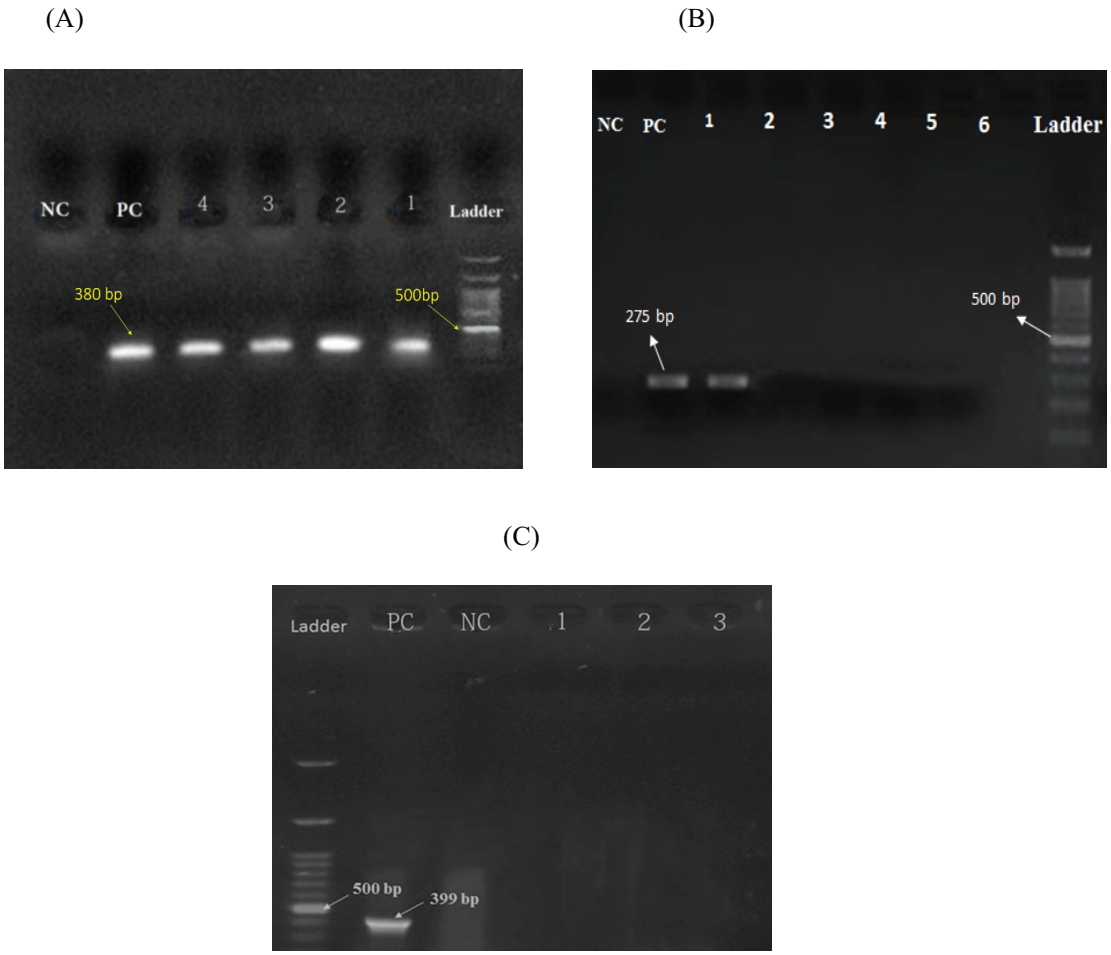

Figure 2: Polymerase chain reaction amplification of bla_{VIM}, bla_{IMP}, and bla_{KPC} *K. pneumoniae* Isolates. **(A):** Lanes 1–4, PCR product of bla_{VIM} (380 bp), **(B):** lane 1–6, PCR product of bla_{IMP} (275 bp), **(C):** lane 1–3, PCR product of bla_{KPC} (399 bp), *K. pneumoniae* ATCC BAA-1705 (positive control), *K. pneumoniae* ATCC BAA-1706 (negative control).

4. Discussion

Klebsiella pneumoniae is responsible for hospital-acquired infections and has recently become one of the most important healthcare-associated infections in hospitals [13]. Several outbreaks of nosocomial infections caused by *K. pneumoniae* have been reported throughout Europe, the United States and Asia [14, 15]. Infection caused by this bacterium often leads to significant mortality and morbidity. Carbapenems with a broad spectrum of activity are considered as the last-line agents for treating infections caused by *K. pneumoniae* [16]. Resistance to carbapenems can be acquired through mechanisms such as drug efflux, loss of porins, and carbapenemase-production [17], the latter of which is predominantly caused by the serine-carbapenemases such as *K. pneumoniae* carbapenemase (KPC) and oxacillinase β-lactamase (OXA), or metallo-β-lactamases including Verona integron-encoded metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM), or imipenemase metallo-β-lactamase (IMP) [18]. bla_{KPC} is the most common carbapenemase in the United States, Europe, Asia, and South America [19–22]. KPC-producing *K. pneumoniae* isolates have been reported in Tehran, Iran [23].

bla_{VIM} and bla_{IMP} have been described in Asia, Europe, North America, South America, and Australia [24, 25]. According to a study by El-kazzaz et al. in 2015, metallo-β-lactamase-producing strains were found in 27% of their isolates, with prevalence rates of 60% and 0% for bla_{VIM} bla_{IMP}, respectively; Acinetobacter strains were the most common MBL-producing strains in their study, and a higher incidence of MBL production was reported than for the *Pseudomonas aeruginosa* and *K. pneumoniae* strains. *P.aeruginosa* may be intrinsically or acquired resistant to antibiotics due to the permeability barrier of the cell surface, multidrug efflux pumps, and production of β-lactamases. According to a study carried out by El-kazzaz, the rate of strains carrying MBL genes was higher than those reported previously. This may be because of an overall increase in the extent of acquiring MBLs genes among strains, which were frequently isolated in their study and showed a high resistance pattern, which is characteristic of their locality. Moreover, the location of MBL genes on class I integron can therefore easily transfer between Gram-negative bacterial isolates [26]. In 2012–2014, Kazmierczak et al. found that 34 (6%) isolates of their study were metallo-β-lactamase-producing *K. pneumoniae* , with a large number of MBL-positive organisms isolated in the Philippines, including 5 unusual species of Enterobacteriaceae and *P. aeruginosa* carrying genes for all three MBL types. The values were higher than reported previously and suggest a strong potential for further spread in diverse geographic regions [27]. In 2011, Lascols et al. showed that the prevalence rates of bla_{VIM} and bla_{KPC} in their clinical isolates were 10% and 34%, respectively; bla_{KPC} showed a high prevalence since the study was conducted in six different hospitals in three countries (Israel, Greece, and the United States) [28]. According to Bratu et al., the KPC gene was found in 24% of carbapenem-resistant *K. pneumoniae* isolates, and the isolates collected in the study were broadly resistant not only to β-lactams, but also to fluoroquinolones and variably to aminoglycosides. Carbapenem-resistant *K. pneumoniae* possessing KPC enzymes appear to be spreading through hospitals in New York City. The outbreak is characterized by the presence of multiple clones, with one dominant strain affecting most hospitals. The presence of this highly resistant clone in most regional hospitals suggests that joint efforts aimed at patient identification and infection control are important for containing the spread of this infection [29]. In a study by weighman et al. in 2015 in Sanjana, Iran, metallo-β-lactamase-producing *K. pneumoniae* strains carrying bla_{IMP} and bla_{VIM} were found at rates of 100% and 6%, respectively. The study showed that most patients were elderly and had urinary tract infections. As expected, females showed a higher prevalence of infection due to ESBL producers than males, as females are more vulnerable to urinary tract infections [30]. In a study by Peymani et al. in Iran, the prevalence of bla_{VIM} and bla_{IMP} were 17.8% and 25%, respectively. The varied range in susceptibility rate of carbapenems among isolates in different studies may be related to the varied antibiotic usage profiles in different geographic regions [31]. In a study by Safari et al. conducted in Iran, no isolates were positive for bla_{VIM}, and some other genes rather than those may be involved in phenotypic production of MBLs and ESBLs and subsequent drug resistance in Hamadan, Iran [32]. In a study carried out by Faghri et al. in Iran, no isolates were positive for bla_{IMP} [33]. Although in studies by Bina et al., Zare et al., Eftekhar et al., and Azimi et al. in Iran, all carbapenemase-producing strains were negative for bla_{KPC}, which may be

because of geographic differences between Iran and other countries, as well as to a reduced susceptibility to at least one extended-spectrum cephalosporin and another mechanism such as of carbapenem resistance resulting from combination of an ESBL or AmpC-type enzyme with porin loss [34, 37]. In our study, the highest and lowest rates of resistance were observed for cefotaxime (98.2%) and gentamicin (43.6%), respectively. A total of 73.2% (134 of 183) of the isolates were positive in MHT, while this test has been shown more than 90% sensitivity and specificity for detecting KPC in the United States [8]. The prevalence of CRKP strains detected in this study is a major concern and thus infection control processes and care measures are needed. The prevalence rates of bla_{VIM}, bla_{IMP}, and bla_{KPC} were 4 (2.18%), 1 (0.5%), and 0, respectively. The number of carbapenem resistant isolates is increasing in Iran. The results of the current study suggest that bla_{VIM}, bla_{IMP}, and bla_{KPC} have a low prevalence in the Kurdistan and Isfahan city, Iran. Thus, other carbapenemase-encoding genes should be evaluated in future studies and PCR should be conducted for detecting all carbapenemase-encoding genes in carbapenem resistant isolates.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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