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Original Article

A multicenter study of β-lactamase-producing *Klebsiella pneumoniae* isolated from university teaching hospitals of Urmia, Iran

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Abstract

Introduction: *Klebsiella pneumoniae* is an opportunistic pathogen accounting for 5-7% of hospital acquired infections. The emergence of carbapenem-resistant *Klebsiella pneumoniae* has been increasing rapidly over recent years causing many therapeutic problems worldwide. This study aimed to research the antimicrobial resistance profile, detect β-lactamase genes among clinical isolates of *K. pneumoniae,* and determine their clonal relatedness.

Methodology: All *Klebsiella pneumoniae* isolates were obtained from teaching hospitals in Urmia, Iran. Antimicrobial susceptibility testing was done by the disk diffusion method. Furthermore, minimum inhibitory concentrations of imipenem were determined by applying Etest strips. Screening of β-lactamase-producing isolates was performed by the combined disk method and modified Hodge test. The detection of β-lactamase genes was conducted by polymerase chain reaction (PCR), and isolates' clonal relatedness was evaluated by random amplified polymorphic DNA (RAPD)-PCR.

Results: Overall, 45 out of 182 (24.7%) *K. pneumoniae* isolates were non-susceptible to imipenem. The combined disk method and modified Hodge test revealed that 93.3% and 71.1% of the imipenem non-susceptible isolates were β-lactamase producers, respectively. The presence of bla_{VIM}, bla_{NDM}, $bla_{KPC,}$ and bla_{IMP} genes was confirmed in 48.9%, 15.6%, 11.1%, and 6.7% of the β-lactamase-producing isolates, respectively. RAPD-PCR revealed that 73% of these isolates were classified into six different clusters.

Conclusions: A relatively high prevalence of β-lactamase genes was seen among multidrug-resistant isolates of *K. pneumoniae*. Most patients infected with β-lactamase-producing isolates had a history of long-term hospitalization and nosocomial infections. The predominance of β-lactamase genes in intensive care unit and internal units alarm clinicians to the growth of hospitalization and mortality rates.

Key words: *Klebsiella pneumoniae*; antimicrobial resistance; metallo-β-lactamase; carbapenemase; nosocomial infection.

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Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a nosocomial opportunistic pathogen that accounts for a variety of infections such as 6-17% of urinary tract infections, 7-14% of pneumonia, 4-15% of septicemia, 2-4% of wound infections, 3-20% of infant septicemia, and 4-17% of infections in intensive care units (ICUs). In general, it is estimated that 5-7% of hospital-acquired infections are caused by this bacterium [1]. Therefore, *K. pneumoniae* is among the important pathogens causing both hospital and community-acquired infections. Moreover, the increasing rate of antimicrobial resistance among *K. pneumoniae* isolates appears to be a major healthcare problem worldwide [2]. Recently, the emergence of extended spectrum β-lactamase (ESBL)-producing *K. pneumoniae* isolates has limited the therapeutic options; therefore, carbapenems remain as the drugs of choice for such infections [3].

The first report of carbapenem-resistant *K. pneumoniae* (CRKP) came from northeast of Scotland in 1997 [4], and the situation became more complicated when various studies from different parts of the world reported CRKP [5-8]. CRKPs are one of the main risk factors for increased mortality and morbidity rates, especially in ICUs. Moreover, only few treatment options are available for these infections [9].

Different mechanisms of carbapenem resistance have been identified including the production of carbapenemases, alteration of the bacterial outer

membrane permeability, and the production of various β-lactamases [6]. According to amino acids sequence homology (Ambler classification system), β-lactamases are divided into the four groups of A, B, C, and D. *K. pneumoniae* carbapenemase (KPC) enzymes are classified as Ambler class A and Bush group 2f. KPC enzymes, primarily found in *K. pneumoniae,* have recently been found in other members of Enterobacteriaceae [10]. Metallo-β-lactamases (MBLs) are kind of β-lactamases belonging to Bush group III and Ambler class B classification systems. MBLs are classified into six groups based on their molecular structure; they include Imipenemase (bla_{IMP}), Verona integron-encoded MBL (bla_{VIM}), Sao Paulo metallo-β-lactamase (bla_{SPM}), German imipenemase (bla_{GIM}), Adelaide imipenemase (bla_{AIM}), and New Delhi MBL (bla_{NDM}) [9].

In recent years, the prevalence of MBLs in Enterobacteriaceae family has been reported frequently [5,6]. A broad range of β-lactam antibiotics (i.e., penicillins, cephalosporins, and carbapenems) is hydrolyzed by MBLs, except for monobactams. Since MBLs-encoding genes are frequently found in integrons, especially class I integrons, they can spread in hospital settings rapidly [7]. Recently, the New Delhi MBLs (bla_{NDM}) were detected in *K. pneumoniae* and *E. coli* isolates; these enzymes confer resistance to aminoglycosides, fluoroquinolones, and other classes of antibiotics. New Delhi MBLs (NDM)-type enzymes were first reported in a patient in Sweden who had a history of hospitalization in New Delhi due to urinary tract infection [11]. Since then, they have been reported in different members of Enterobacteriaceae including *K. pneumoniae*, *Escherichia coli, and Enterobacter* spp. from other parts of the world [11-13].

Various typing methods are being used to characterize clonal relatedness among strains with resistance genes, making it possible to monitor nosocomial infections [14]. A previous study expanded an optimized RAPD-PCR protocol for typing of *K. pneumoniae* strains comparable with pulsed-field gel electrophoresis (PFGE) [15]. The investigation of the relationship between phenotypic and genetic characteristics of multidrug-resistant (MDR) pathogens can be effective in targeting these bacteria in epidemiological studies [16].

Although there are some studies about carbapenemases from different parts of Iran [17,18], we found only limited studies about carbapenemases among *Pseudomonas aeruginosa* isolates in Urmia, Iran, that mostly used phenotypic methods [19,20]. Since a relatively high prevalence of carbapenemases has been reported from this part of the country [21,22], the present study was designed to determine the frequency of different β-lactamases genes and investigate the clonal relationship of β-lactamase-producing isolates of *K. pneumoniae* strains recovered from different teaching hospitals of Urmia, Iran.

Methodology

Bacterial isolates

From December 2013 to September 2016, all *K. pneumoniae* isolates were collected from outpatients and hospitalized patients in four teaching hospitals (i.e., A, B, C, and D), Urmia, Iran. Bacterial isolates were obtained from different clinical specimens including urine, sputum, tracheal discharges, blood cultures, wound discharge, and stool. The bacterial isolates were identified through standard microbiological tests [23]. This study was approved by the Ethics Committee of Urmia University of Medical Sciences (No. IR.umsu.rec.1392.145).

Antimicrobial susceptibility testing

Susceptibility to 11 antimicrobial agents was assessed by using the disk diffusion method (Kirby-Bauer) on Muller-Hinton agar (Merck Co., Darmstadt, Germany) according to the clinical and laboratory standards institute (CLSI) recommendations. The following antibiotic disks (Mast Diagnostics, Bootle Merseyside, UK) were used for antibiogram testing: imipenem (10 µg), ertapenem (10 µg), aztreonam (10 µg), cefepime (30 µg), ceftazidime (30 µg), kanamycin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), cefotaxime (30 µg), amikacin (10 µg), and tobramycin (10 µg). *K. pneumoniae* ATCC 10031 and *Escherichia coli* ATCC 25922 were used as quality control isolates in antimicrobial susceptibility testing [24].

Minimum inhibitory concentration (MIC)

The MIC values for imipenem were determined for all imipenem non-susceptible (resistance+intermediate) *K. pneumoniae* isolates using Etest strips (Liofilchem, Roseto Degli Abruzzi, Italy) according to the CLSI guidelines. The imipenem Etest strips concentrations ranged from 0.002 to 32 mg/L. The MIC values of the isolates were read where the inhibition ellipses intersected the Etest strips. The results were interpreted based on 2016 CLSI criteria [24].

Screening of β-lactamase and carbapenemase-producing isolates

Imipenem non-susceptible isolates of *K. pneumoniae* were examined by two β-lactamase

screening tests: imipenem-EDTA combined disk test (CDT) for metallo beta-lactamases [25] and carbapenemase detection by the modified Hodge test (MHT). MHT was performed according to the CLSI recommendations [24]. Moreover, *K. pneumoniae* ATCC BAA-1705 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive and negative controls, respectively.

DNA extraction and PCR for detection of β-lactamase genes

Bacterial DNA was extracted from the bacterial strains by using a DNA extraction kit (Yekta Tajhiz Azma Co., Tehran, Iran). PCR assays were carried out using specific primers (Bioneer, Daejeon, Korea) described previously for MBL and carbapenemase genes [9,13,17,26-30].

The characteristics of primers used in this study are shown in Table 1. PCR master mix consisted of a 10× PCR buffer with 1× final concentration, $MgCl_2$ (50 mM) with a final concentration of 10 mM, dNTP Mix (10 mM) with 2 mM final concentration (Yekta Tajhiz Azma Co., Tehran, Iran), and forward and reverse primers with 10 μM final concentration [13,17]. PCR amplification was performed in a total volume of 25 microliter (μL) (23 μL of PCR Master Mix plus 2 μL of template DNA). In each run of PCR, positive control was used for each type of the genes.

PCR amplification conditions consisted of the following steps: initial denaturation step at 95°C for 4 minutes followed by 35 cycles of 60 seconds at 94°C (denaturation), 60 seconds at the desired annealing temperatures (Table 1), and 45 seconds at 72°C (extension) with a final extension at 72°C for 7 minutes (Bioer Co. LTD, XP cycler, Hangzhou, China). PCR products were examined by electrophoresis in 1.2% agarose gel containing 1 μL of DNA safe stain in a Tris/Borate/EDTA (TBE) buffer at 90 volts. The gel was visualized by a gel documentation system (SYNGENE, Cambridge, United Kingdom) and photographed. Finally, the results were confirmed by direct sequencing and comparison with the NCBI database.

Molecular typing of β-lactamases-producing isolates

RAPD-PCR was used for studying the clonal relatedness of imipenem non-susceptible isolates of *K. pneumoniae* using RAPD-640, RAPD-7, and AP-4 primers, as previously described by Ashayeri-Panah *et al.* [15]. Thermal cycler program consisted of an initial denaturation step at 94°C for 4 minutes, followed by 50 cycles of denaturation at 94°C for 60 seconds, annealing at the desired temperature for 60 s, extension at 72°C for 120 s, and a final extension at 72°C for 10 minutes. PCR products were analyzed by electrophoresis in 2% agarose gel in Tris-acetate-EDTA (TAE) buffer at 100 volts for 1 h and 20 minutes. The gel was photographed and visualized in a gel documentation system. The clonal relatedness of the isolates was evaluated by the presence or absence of specific bands. Finally, the banding pattern was analyzed both visually and using an online software (http://insilico.ehu.es/dice_upgma/index.php). A dendrogram was built by employing dice comparing methods; moreover, clustering was performed by applying the unweighted pair group method with arithmetic (UPGMA) mean method. Clustering was determined by the cutoff value of 80%, as proposed previously [7,31].

Table 1. Names, sequences, annealing temperatures, and expected sizes for primers used in current study.

Primer	Primer sequence (5'-3')	Annealing Temp°C	Fragment size (bp)
bla$_{KPC}$-F	ATG TCA CTG TAT CGC CGT CT	48	650
bla$_{KPC}$-R	TTT TCA GAG CCT TAC TGC CC		
bla$_{IMP}$-F	CTA CCG CAG CAG AGT CTT TGC	48	587
bla$_{IMP}$-R	GAA CAA CCA GTT TTG CCT TAC C		
bla$_{VIM}$-F	GAT GGT GTT TGG TCG CAT A	60	390
bla$_{VIM}$-R	CGA ATG CGC AGC ACC AG		
bla$_{NDM}$-F	GGT TTG GCG ATC TGG TTT TC	60	621
bla$_{NDM}$-R	CGG AAT GGC TCA TCA CGA TC		
bla$_{GIM}$-F	AGAACCTTGACCGAACGCAG	56	746
bla$_{GIM}$-R	ACTCATGACTCCTCACGAGG		
Bla$_{spm}$-F	GCG TTT TGT TTG TTG CTC	56	786
bla$_{spm}$-R	TTG GGG ATG TGA GAC TAC		
AP4	TCA CGA TGC A	34	Variable
P640	CGT GGG GCT C	34	Variable
RAPD7	GTG GAT GCG A	32	Variable

Statistical analysis

All the data are presented as means ± standard deviation (SD) for quantitative variables and percentages for categorical variables. Statistical analyses were performed by applying SPSS software (SPSS17 Inc., Chicago, IL, USA).

Results

Bacterial isolates

In total, 182 clinical isolates of *K. pneumoniae* were collected from four teaching hospitals (i.e., A: Imam, B: Motahari, C: Taleghani, and D: Shohada) in Urmia, Iran. The majority of the isolates were obtained from Hospital A (n = 83, 45.6%), followed by Hospital D (n = 43, 23.6%), Hospital B (n = 30, 16.5%), and Hospital C (n = 26, 14.3%). In addition, 107 (58.8%) isolates were recovered from outpatients and 75 (41.2%) isolates from hospitalized patients. The isolates were recovered from different clinical samples, with urine being the most common source (137, 75.3%). The frequencies of the other specimens were as follows: sputum 26 (14.3%), wound discharge 10 (5.5%), blood culture 8 (4.4%), and stool culture 1 (0.5%).

Antimicrobial susceptibility testing and MIC determination

The antibiotic susceptibility testing of the isolates revealed that the majority of the isolates were susceptible to imipenem (n = 137, 75.3%), meropenem (n = 131, 72%), and amikacin (n = 121, 66.5%) (Table 2). Also, 92 (50.5%) isolates belonged to MDR isolates, and 24 (53.3%) imipenem-resistant strains were isolated from sputum and the majority of these isolates were recovered from patients hospitalized in internal units (45.7%) and ICUs (22.2%). The MICs of imipenem non-susceptible isolates to imipenem were ≥ 32 mg/L.

Phenotypic detection of β-lactamase and carbapenemase-producing isolates

In general, 42 out of 45 (93.3%) imipenem non-susceptible isolates were positive by CDT, while 32 (71.1%) isolates showed positive results by the MHT method. Also, 43 (95.6%) β-lactamase-producing isolates belonged to hospitalized patients, with the dominance of internal wards (n = 18, 40%) and ICUs (n = 14, 31.1%), while only two β-lactamase-producing isolates were recovered from outpatients. The majority of the β-lactamase-producing isolates were recovered from sputum (n = 24, 53.3%), followed by urine (n = 19, 42.2%) and wound discharge (n = 2, 4.4%).

Detection of β-lactamase genes by PCR

The PCR results confirmed the presence of bla_{VIM}, bla_{NDM}, $bla_{KPC,}$ and bla_{IMP} in 22 (48.9%), 7 (15.6%), 5 (11.1%), and 3 (6.7%) imipenem non-susceptible isolates, respectively. The bla_{SPM} and bla_{GIM} metallo-β-lactamase genes were not found in this study. Also, 1 (2.2%) isolate was positive for both bla_{NDM} and bla_{KPC}, and one (2.2%) isolate carried bla_{VIM}, $bla_{IMP,}$ and bla_{KPC} genes simultaneously.

RAPD-PCR analysis

The RAPD-PCR analysis revealed that P-640 primer had a higher discriminatory power among the isolates than the RAPD-7 and AP-4 primers. The size of RAPD-PCR products varied from 100 bp to 2000 bp, and the number of bands varied from 0 to 13.

Bacterial isolates with less than three bands (n = 8) were excluded from the final analysis; accordingly, the RAPD-PCR dendrogram was constructed for 37 *K. pneumoniae* isolates. The majority of the isolates (n = 27, 73%) showed more than 80% similarity and clustered into six different clones (A to F), while 27% (n = 10) of the isolates had identical patterns (Figure 1). Clone D had a high occurrence (13 isolates) and clones

Table 2. The results of antibiotic susceptibility testing of *K. pneumoniae* isolates.

Antibiotic	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Imipenem	137	75.3	2	1.1	43	23.6
Meropenem	131	72	5	2.7	46	25.3
Amikacin	121	66.5	11	6	50	27.5
Ertapenem	120	65.9	2	1.1	60	33
Tobramycin	102	56	3	1.7	77	42.3
Gentamicin	100	54.9	5	2.7	77	42.3
Ciprofloxacin	97	53.3	9	4.9	76	41.8
Kanamycin	88	48.4	6	3.2	88	48.4
Cefepime	86	47.3	7	3.8	89	48.9
Aztreonam	79	43.4	3	1.7	100	54.9
Cefotaxime	76	41.8	-	-	106	58.2

C and F had low occurrences (two isolates). It is worth noting that 66.7% of the clustered isolates possessed at least one type of the β-lactamase genes under study. Furthermore, 74.1% of the isolates were obtained from the ICU and internal wards of Hospital A. In cluster D containing 13 isolates with ≥ 80% similarity, 12 isolates were collected from Hospital A and only one isolate was collected from Hospital D. These strains belonged to different wards of Hospital A including ICU and internal, kidney transplant, and trauma wards. More than 76.9% of these isolates were recovered in a similar period of two months. The detailed properties of all the clustered isolates are presented in Table 3. Overall, RAPD data analysis indicated that this method could be useful in identifying bacterial clonality in hospital settings.

Finally, 34 (75.6%) patients infected with imipenem non-susceptible isolates confirmed to be infected with at least one type of hospital-acquired infection, 22.2% (n = 10) of whom died of nosocomial infections during the study period. Two and four of the expired patients belonged to clones B and D, respectively.

Figure 1. Dendrogram of 37 β-lactamases producing isolates of *K. pneumoniae* constructed by UPGMA method (A to F clustered isolates with more than 80% similarity). Twenty-seven (73%) isolates showed more than 80% similarity and clustered into six different clones (A to F), while 10 (27%) of isolates had identical patterns.

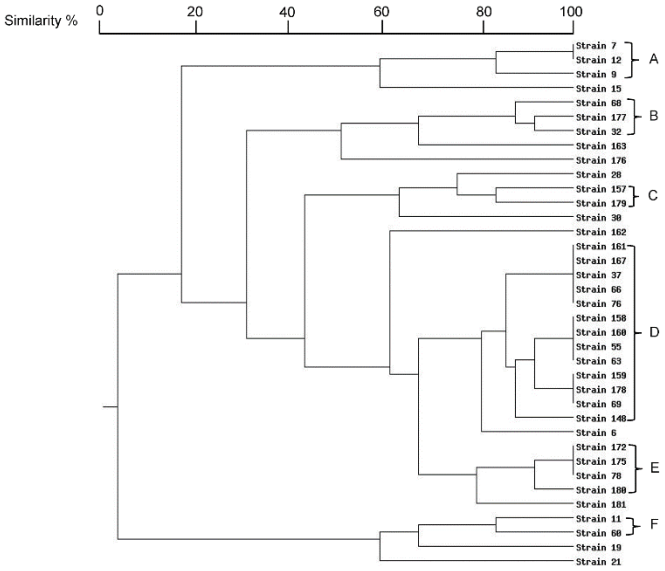

Table 3. Characteristics of clustered isolates (≥ 80% similarity) of *K. pneumoniae.*

Clone	Isolate No.	Specimen	Ward	Hospital	MHT*	*bla*VIM	*bla*IMP	*bla*KPC	*bla*NDM
A	7	Sputum	Internal	Imam	+	-	+	-	-
	12	Sputum	ICU	Imam	+	+	-	-	-
	9	Sputum	ICU	Imam	+	-	-	-	-
B	68	Sputum	ICU	Imam	+	+	-	-	-
	177	Sputum	Internal	Imam	+	-	-	+	-
	32	Sputum	Internal	Imam	+	+	-	-	-
C	157	Sputum	ICU	Imam	+	-	-	-	-
	179	Urine	Kidney Transplant	Imam	-	-	-	-	+
D	161	Sputum	ICU	Imam	+	+	-	-	-
	167	Urine	Kidney Transplant	Imam	-	-	-	-	+
	37	Urine	Internal	Imam	+	+	-	-	-
	66	Sputum	Internal	Imam	+	-	-	-	-
	76	Sputum	Internal	Shohda	+	+	-	-	-
	158	Urine	Trauma	Imam	+	+	-	-	-
	160	Urine	Trauma	Imam	+	-	-	-	-
	55	Sputum	Internal	Imam	+	+	-	-	-
	63	Sputum	Internal	Imam	+	-	-	-	-
	159	Urine	Trauma	Imam	-	-	-	-	-
	178	Sputum	Internal	Imam	-	-	-	-	-
	69	Sputum	ICU	Imam	+	+	-	-	-
	148	Wound discharge	ICU	Imam	+	-	-	-	+
E	172	Urine	Kidney Transplant	Imam	-	+	-	+	+
	175	Sputum	Internal	Imam	-	-	-	-	-
	78	Sputum	ICU	Taleghani	+	+	-	-	-
	180	Urine	Kidney Transplant	Imam	-	-	-	-	+
F	11	Sputum	ICU	Imam	+	+	+	+	-
	60	Sputum	Internal	Imam	+	-	-	-	-

*MHT: Modified Hodge test.

Discussion

Recently, the emergence of CRKP has become a rising problem worldwide. Carbapenems are considered the last therapeutic agents for the treatment of MDR Gram-negative bacteria because of their low toxicity and stability against ESBL and AmpC enzymes [9]. To our knowledge, this is the first attempt to characterize different β-lactamase genes by phenotypic and genotypic methods among clinical CRKP collected from teaching hospitals affiliated to Urmia University of Medical Sciences in northwestern Iran.

In the present study, imipenem with 75.3%, meropenem with 72%, and amikacin with 62.5% susceptibility were the most effective antibiotics against *K. pneumoniae* isolates, whereas a high resistant rate was observed to cefotaxime (58.2%), aztreonam (54.9%), and cefepime (48.9%). There are various reports regarding the antimicrobial resistance profiles of *K. pneumoniae* isolates from different parts of Iran, some of which are consistent with those in the present study [32,33]. However, in some other studies, a higher rate of antimicrobial resistance was stated [3,18]. The mean rate of resistance to imipenem varies from 0 to 54% in different investigations from various parts of Iran [18,34,35]. The diversity of antimicrobial resistance can be attributed to differences in geographical regions the concerning strict rules in antibiotics prescriptions, type of infection, and antibiotic rotation strategies in hospital wards [10]. Bacterial properties such as biofilm formation, β-lactamase production, the presence of mucoid capsules, the anatomic site of bacterial isolation such as wound discharge or catheter cutlers may also alter antimicrobial resistance patterns [36].

The production of different types of β-lactamases is the main cause of carbapenem resistance among clinical isolates of *K. pneumoniae* [6]. We found that 97.8% of the imipenem non-susceptible isolates showed positive results in the phenotypic tests. At present, MHT is the only phenotypic test approved by the CLSI for the detection of carbapenemase-producing isolates [24]. Some studies, however, have reported false positive and false negative results for phenotypic tests [37,38]. The sensitivity of MHT is almost 100%, but its specificity decreases, especially when AmpC β-lactamase and ESBL (CTX-M type) producing isolates or *Enterobacter* species are tested [8,38]. In total, 71.1% of the imipenem non-susceptible isolates in the present study harbored one type of β-lactamase genes (see results). The lack of consistency between phenotypic and genotypic tests may be the consequence of false positive and false negative results in screening methods, the interference of unknown or other types of β-lactamases, or the existence of other carbapenem-resistant mechanisms [6,38]. Among the different types of β-lactamase genes reported from other parts of Iran, the bla_{VIM} and bla_{IMP} MBLs were the most prevalent ones. The frequency of bla_{VIM} among *K. pneumoniae* isolates was as follows: 3.6% in Tehran [17], 10.3% in Isfahan [39], 30% in Babol [40], and 41.6% in Zanjan [41]. In our study, the frequency of bla_{VIM} gene was 48.9%.

In the case of the bla_{IMP} MBL in CRKP isolates, we found only two reports from Iran reporting 3.4% in Isfahan [40] and 100% in Zanjan [41]. In the present study, 6.7% of the isolates were positive for bla_{IMP}. We found that 11.1% of the CRKP isolates were positive for the bla_{KPC} gene. A few molecular studies have confirmed that bla_{KPC} and bla_{NDM}-positive *K. pneumoniae* are found in this part of the world. The first report of bla_{KPC}-harboring isolates of CRKP (2.38%) was from Tehran [39]. Moreover, 11.6% of the CRKP isolates in Kashan [35] were positive for bla_{KPC}.

We found that 7 (15.6%) isolates of CRKP were positive for the bla_{NDM} β-lactamase gene. There are limited published data on the frequency of bla_{NDM} in Iran. Shahcheraghi *et al.* reported the first isolate of bla_{NDM}-positive CRKP (1.1%) [42]. Other studies on bla_{NDM} showed the rates of 12.2% [3] and 8.5% [43] in CRKP isolates. *K. pneumoniae* is the indicator species for resistance determinants; the high frequency of these genes in other Enterobacteriaceae *and Pseudomonas* and the probability of the horizontal transfer of resistance genes in hospital settings might also play a role in this phenomenon [21,42]. We did not find any bla_{SPM} or bla_{GIM} genes in CRKP isolates. This is in agreement with other studies reporting that these genes are confined to distinct geographical regions such as Brazil and Germany [27,44]. Various frequencies of β-lactamase genes could be interpreted by differences in the presence of many subtypes, the use of different pairs of primers, hospitalization wards, and the implementation of hospital infection control strategies. The presence of other bacterial isolates harboring the transferable elements such as integrons and plasmids containing the resistance genes may also contribute to this process.

Some studies have shown that RAPD-PCR could be useful in the differentiation of *K. pneumoniae* isolates in comparison to the gold standard methods such as pulsed field gel electrophoresis [15,26,45]. In this study, RAPD-PCR confirmed that 73% of the CRKP isolates were clonally related, which may indicate an

ongoing transmission cycle in hospital environments, especially among isolates harboring the bla_{VIM} MBL gene collected from Imam Hospital of Urmia, Iran.

Conclusion

We found that imipenem was the most active antibiotic against β-lactamase-producing isolates. Furthermore, the bla_{VIM} and bla_{IMP} MBLs genes were prevalent in this region. To the best of our knowledge, this is the first report of bla_{NDM} and bla_{KPC}-positive CRKP isolates from Urmia, Iran. More than 95% of the genotypically positive isolates were resistant to all the carbapenem antibiotics. All the isolates were MDR, and the majority of the strains were isolated from ICUs and internal wards, a phenomenon that could result in great therapeutic problems. Although PCR is a sensitive, reliable technique for the detection of different types of β-lactamases, due to various and perhaps some new types of these enzymes, it is suggested that both phenotypic and genotypic tests be used in order to obtain reliable results. Finally, the application of simple typing methods such as RAPD-PCR can reveal the clonal relationship between isolates and may lead to the improvement of infection control programs.

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