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Major Article

Molecular detection of β-lactamase and integron genes in clinical strains of *Klebsiella pneumoniae* by multiplex polymerase chain reaction

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Abstract

Introduction: Infections caused by β-lactamase-producing gram-negative bacteria, such as *Klebsiella pneumoniae*, are increasing globally with high morbidity and mortality. The aim of the current study was to determine antimicrobial susceptibility patterns and the prevalence of antibiotic resistance genes (β-lactamase and integron genes) using multiplex PCR. **Methods**: One-hundred *K. pneumoniae* isolates were collected from different clinical samples. Antibiotic susceptibility testing was performed with thirteen different antibiotics. Multiplex-PCR was used to detect β-lactamase (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC}) and integron genes (*int* I, *int* II, and *int* III). **Results:** The highest and lowest rate of resistance was exhibited against amikacin (93%) and imipenem (8%), respectively. The frequency of β-lactamase-positive *K. pneumoniae* was 37%, and the prevalence of the bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC} genes was 38%, 24%, 19%, 12%, 6%, 11%, 33%, 0%, 28%, and 23%, respectively. Of the 100 isolates, eight (8%) were positive for class I integrons; however, class II and III integrons were not detected in any of the strains. **Conclusions:** These results indicate co-carriage of a number of β-lactamase genes and antibiotic resistance integrons on the same plasmids harboring multi-drug resistance genes. It seems that these properties help to decrease treatment complications due to resistant bacterial infections by rapid detection, infection-control programs and prevention of transmission of drug resistance.

Keywords: *K. pneumoniae*. β-lactamase. Integrons. Drug resistance. Multiplex PCR.

INTRODUCTION

Klebsiella pneumoniae is an important causative agent of both hospital-acquired and community-acquired infections such as pneumonia, urinary tract infections, meningitis, and septicemia[1]. Multi-drug resistant (MDR) strains can be quite problematic, especially for elderly or immunocompromised patients and infants with an immature physiology[2]. Release of β-lactamases is a significant resistance mechanism against antimicrobial agents[3]. β-lactamase-producing *K. pneumonia* can degrade a wide range of β-lactam antibiotics such as penicillins, carbapenems, cephalosporins, and cephamycins[2,4]. These enzymes can be divided into four classes (A, B, C, and D) based on the Ambler classification. The temoneira (TEM), cefotaximase (CTX-M), sulfhydryl variable (SHV), Vietnam extended-spectrum β-lactamase (VEB), *Pseudomonas* extended-*resistant* (PER), and Guiana extended-spectrum (GES) enzymes belong to class A; the Verona integron-encoded metallo-β-lactamase (VIM), imipenem (IMP), and *K. pneumoniae* carbapenemase (KPC) enzymes belong to class B; and oxacillin hydrolyzing enzyme (OXA) is classified as class D according to the Ambler classification[5-7]. Researchers have reported that the incidence of β-lactamase-producing *K. pneumonia* ranges from 6 to 88% at different health care locations[8]. Bla_{SHV} β-lactamases are related to high level ceftazidime resistance, but not to cefazolin or cefotaxime resistance, while bla_{CTX-M} β-lactamases are more effective against cefotaxime. In contrast, TEM β-lactamases confer resistance against oxyimino-β-lactams groups such as ceftazidime, cefotaxime, and aztreonam. In addition to β-lactamase encoding plasmids, transportable genetic elements such as integrons can also contribute to the evolution and distribution of MDR genes (bla_{TEM}, bla_{CTX-M}, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC}) in *K. pneumoniae* by vertical or horizontal transmission[9,10]. Five classes of integrons have been proposed based on the amino acid sequences of Int I proteins. Three classes of antibiotic

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resistance integrons (ARIs; I, II, and III), identified based on particular integrase genes[11], are usually associated with MDR phenotypes. The transportable class I integron is related to transposon Tn21 and is commonly observed in β-lactamase-producing clinical isolates of *K. pneumonia*[12]. Class II integrons are detected less frequently in bla_{KPC}-producing bacteria, such as *K. pneumoniae* and *Escherichia coli*, and class III integrons are detected quite infrequently in β-lactamase-producing *K. pneumoniae*[13]. Previous reports have demonstrated the production of various β-lactamases, such as bla-ESBL, and resistance to several antibiotics groups via ARI gene carriage in clinical isolates of *K. pneumonia*[14]. Unfortunately, the incidence of β-lactamase-producing *K. pneumoniae* is on the rise[15]. The detection of different β-lactamase genes in resistant bacteria and characterization of their antimicrobial susceptibility profiles could provide important data regarding high risk factors and infection epidemiology[16-18]. To date, only a few studies have investigated the types of β-lactamase-producing *Enterobacteriaceae* and strains possessing integrons present in Iranian hospitals[19,20]. Thus, the aim of the present study was to determine the prevalence of bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC}, as well as *int* genes (I, II and III) in clinical *K. pneumonia* strains isolated from two large urban university general hospitals in Tehran, Iran using multiplex-polymerase chain reaction (M-PCR).

METHODS

This cross-sectional study was conducted from April 2014 to March 2015, at two teaching hospitals in Tehran, Iran. One hundred non-repetitive *K. pneumonia* isolates were obtained from different clinical specimens including blood, skin lesions, broncho-alveolar lavage (BAL), urine, sputum, cerebrospinal fluid (CSF), pus, pleural effusion, ascites, and catheter specimens. Each sample was cultured on MacConkey agar (Merck, Darmstadt, Germany) and incubated at 37°C for 24h. Resulting colonies were identified as *K. pneumonia* using standard biochemical and microbiological tests, including urease, oxidase, motility, citrate utilization, Triple sugar iron agar (TSI), Methyl Red-Voges Proskauer (MR-VP), and Sulfide Indole Motility (SIM), and were further confirmed with the API 20E system (Analytab, Inc., New York).

Antibiotic susceptibilities were determined using the disc diffusion method on Mueller-Hinton Agar (Merck Co., Germany) plates in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for the following antibiotics (Mast, Merseyside, UK): amoxicillin/clavulanate (AUG; 20/10µg), ciprofloxacin (CIP; 5µg), amikacin (AK; 30µg), trimethoprim-sulfamethoxazole (TS; 2.5µg), cefotaxime (CTX;30µg), Ampicillin (AMP; 10µg), aztreonam (AZT; 30µg), imipenem (IPM; 10µg), gentamicin (GEN; 10µg), ceftazidime (CAZ;30µg), cefepime (FEP; 30µg), ceftriaxone (CRO; 30µg), imipenem (IMP; 10µg), and levofloxacin (LEV; 5µg). Briefly, a bacterial suspension was obtained from fresh cultures. The turbidity of each bacterial suspension was adjusted to a value equivalent to the no. 0.5 McFarland turbidity standard and then cultured on Mueller-Hinton agar (Oxoid, UK). The zone of inhibition diameter was measured following incubation at 37°C for 18-24 hours; the results were reported as susceptible, intermediate, and resistant. *K. pneumoniae* ATCC1029 was used as the quality control[14].

Multiplex-PCRs were performed to detect β-lactamase genes (bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC}) and *int* genes (I, II, and III) using a master cycler gradient (Eppendorf Co., Germany). Genomic deoxyribonucleic acid (DNA) was extracted from *K. pneumoniae* colonies grown overnight on blood agar (Merck Co., Germany) plates using the boiling method[14]. Briefly, a loopful of bacteria from a colony was suspended in 700µl sterile distilled water, boiled for 10 min, centrifuged at 7,000×*g* for 4 min at 4°C, cooled on ice for 10 min, and then centrifuged for 3 min at 8,000×*g*. The concentration and quality of the extracted cellular DNA were assessed using a Nanodrop spectrophotometer (ND-1,000; Thermo Scientific; Wilmington, DE, USA). The β-lactamase and integron genes were amplified by M-PCR using specific primers detailed in **Table 1.** M-PCR was carried using 1.5µl of extracted genomic DNA in a 25µl PCR reaction mixture consisting of 2.5µl 10× PCR buffer, 1.5µl $MgCl_2$ (50mM), 0.5µl dNTPs (10mM), 1.5µl of each primer, 0.5µl of Taq DNA polymerase (5U/µl; Amplicon Co., Denmark), and 15.5µl sterile distilled water. M-PCR was performed under the following conditions: denaturation at 94°C for 1 min; 35 cycles of denaturation at 94°C for 30s, annealing at 59°C for 30s, and extension at 72°C for 1 min; and a final extension at 72°C for 6 min. For amplification of the *int* genes (I, II, and III), the reaction mixture was amplified using a thermal gradient cycler (Eppendorf Co., Germany) with the following PCR protocol: one cycle of 5 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 65°C, and 1 min at 72°C; and one cycle of 10 min at 72°C[14].

RESULTS

One-hundred *K. pneumoniae* isolates were obtained from 374 (26.7%) different clinical specimens. Specimens included blood (n=7, 7%), skin lesions (n=9, 9%), BAL (n=5, 5 %), urine (n= 62, 62%), sputum (n= 6, 6%), CSF (n=3, 3%), Pus/swap (n=2, 2 %), pleural effusion (n=1, 1%), ascites (n=2, 2%), and catheter (n= 3, 3%) samples. Distribution analysis of the *K. pneumoniae* strains showed that most (62%) isolates were obtained from urine and the lowest (1%) number was isolated from pleural effusion samples. The mean age of the population studied was 47±1.5 years, with a range of 10 to 76 years. The strains were isolated from patients belonging to various age groups: [(10-25 years; 29), (26-40 years; 38), (41-55 years; 43), (56-60 years; 11), and (60-76 years; 7)]. Sixty-seven (67%) patients were male and 33 (33%) were female.

Antibiotic susceptibility tests using the Kirby-Bauer method showed that the level of resistance to amoxicillin/clavulanate, ciprofloxacin, amikacin, trimethoprim-sulfamethoxazole, cefotaxime, ampicillin, aztreonam, imipenem, gentamicin, ceftazidime, cefepime, ceftriaxone, and levofloxacin was 37%, 37%, 93%, 84%, 52%, 87%, 59%, 8%, 24%, 67%,52%, 43%, and 26%, respectively (**Table 2**). The antibiotic susceptibility profiles of non-β-lactamase-producing and β-lactamase-producing *K. pneumoniae* strains are detailed in **Table 3**.

TABLE 1

Nucleotide sequences of the primers used for M-PCR.

Genes	Oligonucleotide sequence (5′→3′)	Size of amplicon (bp)
bla-SHV	F: 5'-ATGCGTTATATTCGCCTGTG-3' R: 5'-TGCTTTGTTATTCGGGCCAA-3'	747
bla-TEM-1	F: 5'-TCGCCGCATACACTATTCTCAGAATGA-3' R: 5'-ACGCTCACCGGCTCCAGATTTAT-3'	445
bla-CTX-M	F: 5'-ATGTGCAGCACCAGTAAAGTGATGGC-3' R: 5'-TGGGTAAAGTAAGTGACCAGAATCAGCGG-3'	593
bla-PER	F: 5'-AATTTGGGCTTAGGGCAGAA-3' R: 5'-ATGAATGTCATTATAAAAGC-3'	925
bla-KPC	F; 5'-CGTCTAGTTCTGCTGTCTTG -3' R: 5'-CTTGTCATCCTTGTTAGGCG -3'	538
bla-VEB	F: 5'-CGACTTCCATTTCCCGATGC-3' R: 5'-GGACTCTGCAACAAATAC GC-3'	643
bla-GES	F: 5'-ATGCGCTTCATTCACGCAC-3' R: 5'-CTATTTGTCCGTGCTCAGG-3'	860
bla-VIM	F: 5'-GATGGTGTTTGGTCGCATA-3' R: 5'-CGAATGCGCAGCACCAG-3'	390
bla-IMP	F: 5'-CATGGTTTGGTGGTTCTTGT-3' R: 5'-ATAATTTGGCGGACTTTGGC-3'	448
bla-OXA	F: 5′-AGC CGT TAA AAT TAA GCC C-3′ R: 5′-CTT GAT TGA AGG GTT GGG CG-3′	919
intI	F: 5'-GCCTTGCTGTTCTTCTACGG-3' R: 5'-GATGCCTGCTTGTTCTACGG-3'	558
intII	F: 5′-CACGGATATGCGACAAAAAGGT-3′ R: 5′-GTAGCAAACGAGTGACGAAATG-3′	789
intIII	F: 5′-GCCTCCGGCAGCGACTTTCAG-3′ R:5′-ACGGATCTGCCAAACCTGACT-3′	979

M-PCR: multiplex polymerase chain reaction; ***bla-TEM-1***: temoniera β-lactamase-1; ***bla-CTX-M***: cefotaximase; ***bla-SHV***: sulphydril variable β-lactamase; ***bla-PER***: *Pseudomonas* extended resistance; ***bla-KPC***: *Klebsiella pneumoniae carbapenemase*; ***bla-VEB***: *Vietnamese extended spectrum beta-lactamase*; ***bla-GES***: *Guiana Extended Spectrum* β-Lactamases; ***bla-VIM***: Verona imipenemase; ***bla-IMP***: imipenemase; ***bla-OXA***: *oxacilinases*; **Int I**: class I integrons; **Int II:** class II integrons; **Int III**: class III integrons.

TABLE 2

Antibiotic resistance patterns in *Klebsiella pneumoniae* isolates.

Antibiotic	Resistant		Intermediate		Susceptible	
	No	%	no	%	no	%
Amoxicillin/clavulanate (Aug)	37	37.0	0	0.0	63	63.0
Ciprofloxacin (CIP)	37	37.0	5	5.0	58	58.0
Amikacin (AK)	93	93.0	3	3.0	4	4.0
Trimethoprim-sulfamethoxazole (TS)	84	84.0	4	4.0	12	12.0
Cefotaxime (CTX)	52	52.0	1	1.0	47	47.0
Ampicillin (AMP)	87	87.0	2	2.0	11	11.0
Aztreonam(AZT)	59	59.0	1	1.0	40	40.0
Imipenem (IPM)	8	8.0	9	9.0	83	83.0
Gentamicin (GEN)	24	24.0	6	6.0	70	70.0
Ceftazidime (CAZ)	67	67.0	2	2.0	31	31.0
Cefepime (FEP)	52	52.0	0	0.0	48	48.0
Ceftriaxone (CRO)	43	43.0	1	1.0	56	56.0
Levofloxacin (LEV)	26	26.0	0	0.0	74	74.0

TABLE 3

Antibiotic resistance rates of non-β-lactamase-producing and β-lactamase-producing *Klebsiella pneumoniae* strains.

Antibiotic	Non-β-lactamase-producing *Klebsiella pneumoniae* strains (%)	β-lactamase-producing *Klebsiella pneumoniae* strains (%)
Amoxicillin/clavulanate (Aug)	37.0	63.0
Ciprofloxacin(CIP)	21.0	42.0
Amikacin (AK)	18.0	45.0
Trimethoprim-sulfamethoxazole (TS)	32.0	31.0
Cefotaxime (CTX)	14.0	49.0
Ampicillin (AMP)	35.0	28.0
Aztreonam(AZT)	12.0	51.0
Imipenem (IPM)	6.0	57.0
Gentamicin (GEN)	31.0	32.0
Ceftazidime (CAZ)	33.0	30.0
Cefepime (FEP)	24.0	39.0
Ceftriaxone (CRO)	15.0	48.0
Levofloxacin (LEV)	7.0	56.0

The β-lactamase gene amplification test (M-PCR) simultaneously amplified and identified the existence of the target genes and showed that the prevalence of the bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{VEB}, bla_{PER}, bla_{GES}, bla_{VIM}, bla_{IMP}, bla_{OXA}, and bla_{KPC} genes was 38%, 24%, 19%, 12%, 6%, 11%, 33%, 0%, 28%, and 23%, respectively (**Figure 1**). Molecular distribution analysis of the integron genes showed that only 11 (8.6%) of the 100 isolates contained class I integrons; however, class II and class III integrons were not detected in any of the isolates (**Figure 2**).

DISCUSSION

β-lactamase-producing *K. pneumoniae* was first identified in 1983[21]. Most infections caused by *K. pneumoniae* are due to multi-drug resistant strains such as β-lactamase producing isolates[22]. Recent studies have shown that the incidence of β-lactamase-producing *K. pneumoniae* is increasing in several countries such as Iran[22,23], India[24,25], and Italy[26]. Resistance to various antibiotics is related to the existence of transmissible plasmids and integrons, which can be integrated into plasmids or the chromosome[27]. These transmissible elements often contain resistance factors that can be transferred to other microorganisms. In this study, we examined the susceptibility of 100 clinical *K. pneumonia* strains against thirteen antibiotics; high resistance was observed for AK (93%), TS (84%), AMP (87%), AZT (59%), GEN (67%), and FEP (52%). Amiri et al.[28] reported that the resistance to ampicillin, ceftazidime, ceftriaxone, aztreonam, and cefotaxime was 92%, 67%, 65%, 64%, and 59%, respectively in *K. pneumoniae* isolates[28], values similar to the rates reported in this study. Our results indicate that only eight β-lactamase-producing isolates were resistant to imipenem using the disk diffusion method. This high (83%) susceptibility to imipenem is in agreement with the reports of Mansury et al.[29], Ahmad et al.[30], Amiri et al.[28], and Edelstein et al.[31]. Only three *K. pneumoniae* isolates were resistant to all antibiotics tested.

Multi-drug resistant (MDR) strains are defined as strains resistant to three classes of antimicrobial agents[32]; therefore, 31% of our isolates can be classified as MDR. This finding contrasts those reported by Mansury et al.[29]. A total of 52% and 67% of our isolates were resistant to the third generation cephalosporins ceftazidime and cefotaxime, respectively,

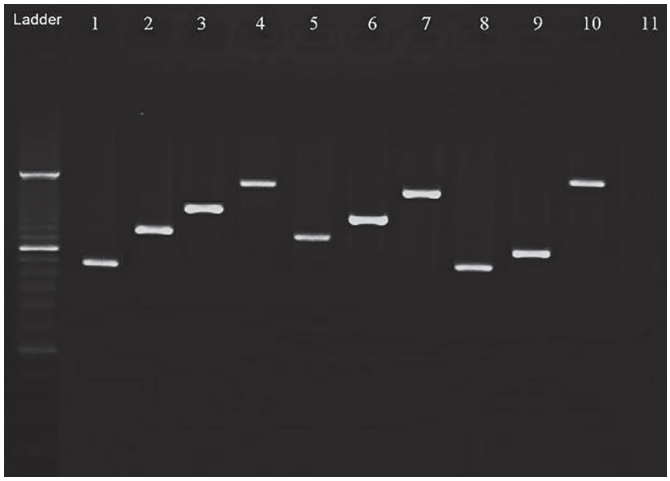

FIGURE 1 - M-PCR amplification of β-lactamase genes in selected *Klebsiella pneumoniae* isolates. Lane 1: *bla-TEM-1* (447bp), **Lane 2**: *bla-CTX-M* (593bp), **Lane 3**: *bla-SHV* gene (747bp), **Lane 4**: *bla-PER* (925bp), **Lane 5**: *bla-KPC* (538bp), **Lane 6**: *bla-VEB* (643bp), **Lane 7**: *bla-GES* (860bp), **Lane 8**: *bla-VIM* (390bp), **Lane 9**: *bla-IMP* (448bp), **Lane 10**: *bla-OXA* (919bp), **Lane 11**: negative control; *Escherichia coli* ATCC 25922, Ladder: 50bp DNA size ladder. **M-PCR**: multiplex polymerase chain reaction; ***bla-TEM-1***: temoniera β-lactamase-1; ***bla-CTX-M***: Cefotaximase; ***bla-SHV***: sulphydril variable β-lactamase; ***bla-PER***: Pseudomonas extended resistance; ***bla-KPC***: *Klebsiella pneumoniae carbapenemase*; ***bla-VEB***: *Vietnamese extended spectrum beta-lactamase*; ***bla-GES***: *Guiana Extended Spectrum* β-Lactamases; ***bla-VIM***: Verona imipenemase; ***bla-IMP***: Imipenemase; ***bla-OXA***: *oxacilinases*; **DNA**: deoxyribonucleic acid.

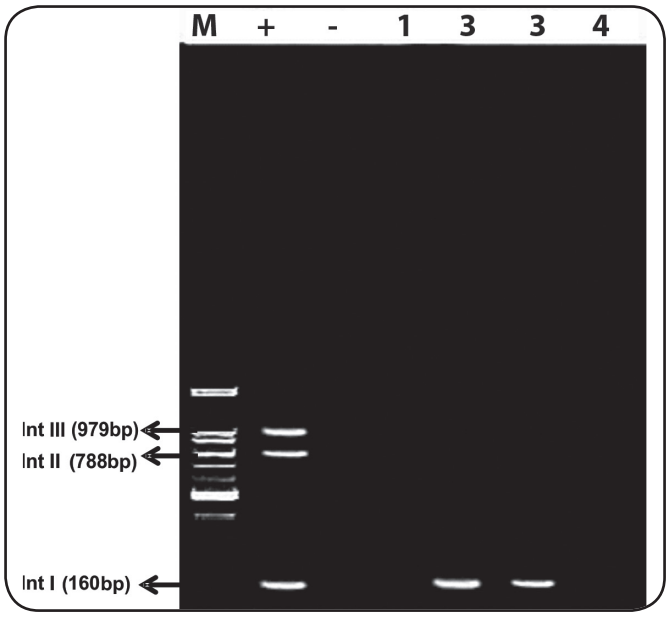

FIGURE 2 - M-PCR amplification of *int* genes in four selected *Klebsiella pneumoniae* isolates. M: 100bp DNA size marker; **Lane +:** quality control (*K. pneumoniae* ATCC 1029); **Lane -:** negative control (*Escherichia coli* ATCC 25922), **Lane 1-4:** M-PCR gene products. **Int I**: class I integrons; **Int II:** class II integrons; **Int III**: class III integrons; **M-PCR**: multiplex polymerase chain reaction**; DNA**: deoxyribonucleic acid.

which is similar to the rates reported by Ullah et al.[33], Amiri et al.[28], and Jalalpoor et al.[34]. Of the MDR isolates, 28 strains were β-lactamase-positive (28%). These results are in agreement with those of Shukla et al.[35] and Sarojamma et al.[36] who reported that 28% and 32% of their strains were β-lactamase producers, respectively[35,36]. The incidence of β-lactamase-producing *Klebsiella* spp. has been reported to vary from 42-44% (in the USA)[37-39], 4.9% (in Canada)[40], 20.8% (in Spain)[41], 28.4% (in Taiwan)[42], 78.6% (in Turkey)[43], 20% (in Algeria)[44], and 51% (in China)[45]. In this study, the highest percentage of β-lactamase-roducing strains was derived from urine samples (14%).

The aim of this study was to determine the prevalence of several β-lactamase and integrin genes (I, II, III) in clinical *K. pneumoniae* isolates. The M-PCR results for each resistance gene were as follows: bla_{TEM} was detected in 37.8% (14.37), bla_{CTX} in 24.3% (9.37), bla_{SHV} in 18.9% (7.37), bla_{VEB} in 10.8% (4.37), bla_{PER} in 5.4% (2.37), bla_{GES} in10.8% (4.37), bla_{VIM} in 8.1% (3.37), bla_{IMP} in 0% (0.37), bla_{OXA} in 27% (10.37), and bla_{KPC} in 24.3% (9.37) of the isolates. Bora et al.[46] reported that of the three β-lactamase genotypes, bla_{TEM} in detected most predominately in β-lactamase-producing *K. pneumoniae* (77.58%)[46]. Monstein et al.[47] detected bla_{SHV} in 8.1% (3.37); bla_{SHV} and bla_{TEM} in 2.7% (1.37); and bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ in 8% (3.37) of their *K. pneumonia* isolates[47]. Hassan and Abdalhamid.[48] reported a very high prevalence of $bla_{CTX\text{-M}}$ (97.4%) in comparison to the prevalence of bla_{SHV} (23.1%) in *K. pneumoniae* strains[48]. However, in Europe, East Asia, and Latin America, as well as in the current study, bla_{TEM}, bla_{CTX}, and bla_{SHV} appear to be the predominant β-lactamase genes in clinical *K. pneumoniae* isolates.

Ahmed et al.[49] reported that the prevalence of bla_{PER} was 22.4%; however Nasehi et al.[20], detected bla_{PER} in only 7.5% of their isolates, which is similar to the results of this study. Borges-Cabral et al.[50], reported that bla_{KPC} was present in 41.7% of their isolates; however, Bina et al.[51] did not observed bla_{KPC} (0%) in any of their strains and the rate in the present study was 23%. Limbago et al.[52] observed the bla_{IMP} gene in all of their clinical *K. pneumoniae* isolates; however, we did not detect this gene in any of our isolates. Udomsantisuk et al.[53] reported that the frequency of the bla_{VEB} gene was 30% among β-lactamase-positive *K. pneumoniae* strains; however, in the current study, the frequncy of bla_{VEB} gene was 12%. Iraz et al.[54] reported that 86% of the carbapenem-resistant *K. pneumoniae* strains carried the bla_{OXA} gene; however, Charrouf et al.[55] found that only 6% of their isolates carried this gene. Our results did not match either of these studies; in our study, the prevalence of this gene was 28%. Psichogiou et al.[56] found that the frequency of the bla_{VIM} gene in clinical *K. pneumoniae* strains was 37.6%, which is consistent with our results (33%). This reflects a significant increase in the prevalence of bla_{TEM} and bla_{VIM} in Iran. In comparison, the major β-lactamase gene found in Arab countries appears to be $bla_{\text{CTX-M}}$[57-59].

In addition to β-lactamase genes, we also evaluated integron gene prevalence in the 100 *K. pneumoniae* isolates. Our results indicate that only eight isolates were positive for class I integrons, while class II and class III integrons were not detected in any of the isolates. This finding is comparable to those of Lima et al.[60] and Ashayeri et al.[61]. Class III integrons have been reported only in very few studies[14,62,63]. Mobarak-Qamsari et al.[64] identified 22 (44%) class I integron-carrying *K. pneumoniae* isolates, but only three (6%) of the isolates had class II integrons and none contained class III integrons[64]. These findings suggest that class I integron genes may play a critical role in the distribution of β-lactamase-encoding genes among clinical β-lactamase-producing *K. pneumoniae* isolates. The increase in multidrug resistance and the underlying mechanisms require further investigation

In conclusions, our study demonstrates that there is a high level of $bla_{\text{TEM,}}$ bla_{VIM}, and class I integrons in the β-lactamase-producing *K. pneumoniae* strains circulating in hospitals in Tehran, Iran. This trend of MDR profiles associated with the presence of $bla_{\text{TEM,}}$ bla_{VIM}, and class I integron genes is worrying. The high prevalence rate of these resistance genes highlights the necessity for establishing a national antibiotic susceptibility surveillance network for monitoring infections due to *Enterobacteriaceae* spp. in Iran. It seems that these properties help to decrease treatment complications and mortality rate due to resistant bacterial infections by rapid detection of β-lactamases genes, infection-control programs and prevention of transmission of drug resistant-strains. A combination therapy can be useful to prevent resistance during therapy resulting in complete remission of patient and resistant infections control. One of the limitations of the present study was that, other β-lactamases family genes and also other antibiotic resistance mechanisms were not assessed due to the financial constraints of molecular and gene tests. So, further investigations are needed to obtain more accurate and effective results.

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Conflict of interest

The authors declare that there is no conflict of interest in the present study.

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