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Original Article

Frequency of Extend Spectrum β-Lactamases Genes among *Klebsiella Pneumoniae* Strains Isolated from Patients Admitted to Shahid Beheshti Hospital, Babol City, Iran

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Abstract

Background: Regarding the emergence and increasing of multidrug-resistant strains among *Klebsiella pneumoniae* nosocomial isolates the therapeutic options for the treatment has been limited. The β-lactamases enzymes are the major defense of gram-negative bacteria against antibiotics. The aim of this study was to the detection of bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes among *K. pneumoniae* strains isolated from patients who admitted to Shahid Beheshti hospital of Babol city, Iran, in 2016. **Materials and Methods:** This study was conducted on 50 samples of *K. pneumoniae* strains isolated from hospitalized patients. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method according to CLSI guidelines. The bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes were detected by polymerase chain reaction method. **Result:** Among studied strains, the prevalence of bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes were 24 (49%), 44 (88%), and 36 (72%), respectively. In this study, imipenem and nitrofurantoin were more effective than other antibiotics. Also, 100% of strains were susceptible to imipenem. **Conclusions:** The prevalence of antibiotic resistance genes detected in this study implies a great concern for the treatment of multidrug-resistant *K. pneumonie*. Hence, infection control measures, including antibacterial management and identification of resistant isolates for preventing of nosocomial outbreaks have become highlighted.

Keywords: *Klebsiella pneumoniae*, β-Lactamases, Multidrug Resistant, PCR

Introduction

Klebsiella pneumoniae is regarded as an opportunistic pathogen that is effectively adapted to the environmental health care units and can lead to a wide range of community and nosocomial acquired infections, such as urinary tract infections, pneumonia, bloodstream infection, pyogenic liver abscess, etc. After *Escherichia coli*, *K. pneumoniae* is considered as the main cause of nosocomial infections among gram-negative bacteria (1). The high prevalence of multidrug-resistant (MDR) strains among nosocomial infections has made it difficult to select the appropriate drug for the treatment of *K. pneumonia* (2). Several virulence factors have been identified in *K. pneumoniae*, the most important of which are capsule and bacterial adhesions including type 1 and 3 fimbriae for binding to host cells (3). TEM, SHV, and CTX-M, OXA are the most important β-lactamases of *K. pneumonia*. *K. pneumoniae* is one of the most prevalent extend spectrum β-lactamases (ESBL)-producing bacteria belonging to family Enterobacteriaceae (4). The ESBL-producing *K. pneumoniae* strains are increasing, while in the 1980s the first strain was described. Currently, ESBL-producing strains have reported from various microorganisms such as *E. coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, etc. (5). Because of the potential transmission of this bacteria at the hospital and the acquisition of antibiotic resistance genes, the level of infections caused by this microorganism has increased and has led to a health problem. Therefore, knowing the pattern of antibiotic resistance and pathogenic factors can help treat (6). This study aimed to evaluate antibiotic susceptibility and detecting the bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes.

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Materials and Methods

Collecting and identifying isolates

This study was conducted on *K. pneumoniae* strain isolates from 50 patients who admitted at Shahid Beheshti hospital among clinical samples including urine (n=37), blood (n=10) and sputum (n=3) over six months in 2016. The *K. pneumoniae* strains were identified and confirmed by standard biochemical and microbiological tests.

Antibiotic susceptibility testing

The antibiotic susceptibility test was performed by the disc diffusion method using Mueller Hinton agar plates according to the CLSI guidelines as follows: imipenem (10µg) , aztreonam (30µg), nitrofurantoin (300µg), ceftazidime (30µg), amikacin (30µg), cefepime (30µg), piperacillin (100µg), ciprofloxacin (5µg), cefotaxime (30 µg), co-trimoxazole (25µg), cefixime (5µg), and ceftriaxone (µg) that obtained from Rosco (Denmark) (7).

DNA extraction and polymerase chain reaction (PCR) identification

The DNA was extracted by commercial Roche kit (Germany) as recommended by the manufacturer. After that, bla_{TEM}, bla_{CTX-M}, and bla_{SHV} genes were detected by specific universal primers (Gene Fanavaran Co, Tehran, Iran). Primers details are listed in Table-1. The PCR steps for each gene amplification were as follow: Initial denaturation step at 95°C for 5 min, denaturation at 94°Cfor 45 s, annealing at 60°C for 30 s for bla_{SHV}, bla_{TEM} and 59°C for bla_{CTX-M}, extension at 72°C for 1 min followed by 30 cycles, then a final extension step at 72°C for 5 min. Finally, the PCR products were electrophoresed on 1% agarose gel.

Result

Among a total of 50 *K. pneumoniae* strains, the prevalence of bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes were 24 (49%), 44 (88%), and 36 (72%), respectively (Figure-1 and 3). In antimicrobial susceptibility assay, imipenem and nitrofurantoin were more effective than other antibiotics, in which 100% of strains were susceptible to imipenem, 60% of strains were resistant to ceftazidime, and 38% of strains were sensitive to cefotaxime (Table-2). On the other hand, 68% and 32% of strains were distinguished as MDR and non-MDR (Table-3).

Discussion

K. pneumoniae is a gram-negative bacteria that play an important clinical role in the hospital developed infections and also a urinary tract infection. The excessive use of antibiotics in recent decades has been led to an increase in the emergence of resistant strains with MDR in gram-negative bacteria such as *K. pneumonia* with the ability to produce different enzymes (8). The ESBL are resistant to several antibiotics such as cephalosporins, penicillins, ciprofloxacin, cefotaxime, etc. The presence and transmission of genes that encoding the beta-lactamase enzymes among gram-negative bacteria have become a major global concern because the ESBL-producing strains are also resistant to other types of antibiotics (9, 10). The results of this study indicate that the imipenem with 100% susceptibility was the most effective antibiotic against *K. pneumonia* strains isolated from clinical specimens that this issue has been proven in similar studies (11, 12). However, in a similar study, the susceptibility of imipenem in *K. pneumonia* was described 94.9% (13). *K. pneumonia* imipenem-resistant strains are reported at a low level in most studies, though the discriminative usage may lead to the emergence of resistant strains to both imipenem and meropenem antibiotics. In the present study, the resistance to cefotaxime, ceftriaxone, and cefixime were reported 58%, 32%, and 30%, respectively. However, the antimicrobial susceptibility pattern was achieved 41% and 37.2% by Eftekhar *et al*. for ceftazidime and ceftriaxone, respectively (14). While in a study conducted in Saudi Arabia in 2009, resistance to ceftazidime was reported 97% (15). Saeidi *et al*. study in Zabol city indicated that the resistance rate for third-generation cephalosporins such as ceftriaxone

Table 1. Specific PCR primers for detection of *SHV*, *TEM* and *CTX-M* genes

Gene name	Primer name	Nucleotide sequence (5'-3')	Amplicon size (bp)
bla_{CTXM}	CTX-M-F	ATGTGCAGYACCAGTAARGTKATGGC	593 bp
	CTX-M-R	TGGGTRAARTARGTSACCAGAAYCAGCGG	
bla_{SHV}	SHV-F	CGCCTGTGTATTATCTCCCT	293 bp
	SHV-R	CGAGTAGTCCACCAGATCCT	
bla_{TEM}	TEM-F	TTTCGTGTCGCCCTTATTCC	403 bp
	TEM-R	ATCGTTGTCAGAAGTAAGTTGG	

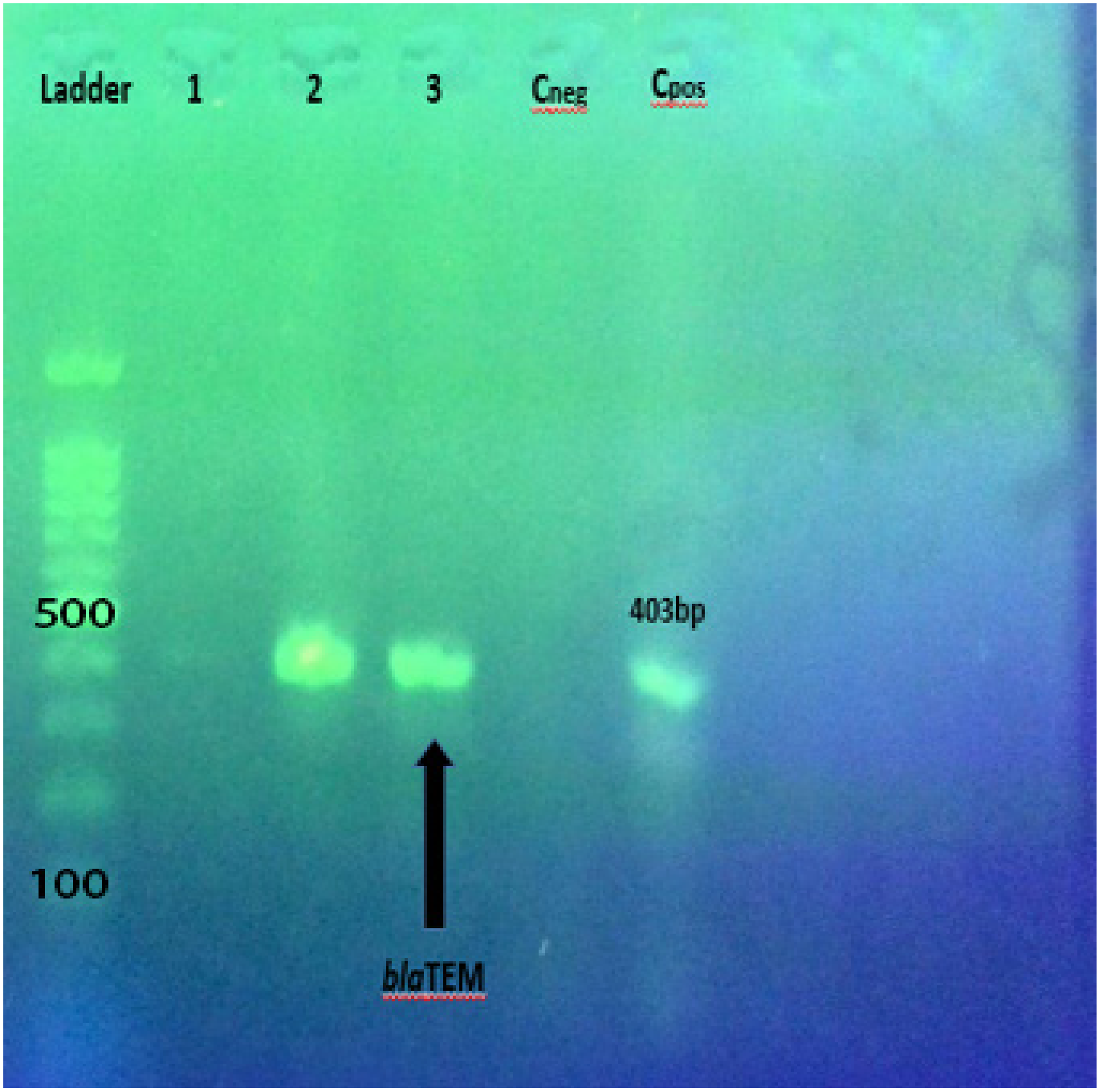

Figure 1. The result of PCR for bla_{TEM} gene.C-pos: Positive control, C-neg: Negative control, Left line: 100bp DNA ladder, Line 1: negative isolate, Line 2 and 3: Positive isolates (403bp).

and ceftazidime was 100% (16). These various results may be due to uncontrolled consumption of antibiotics, the emergence of resistant strains, and therapeutic regimen management by PCR, 44 (88%) and 36 (72%) of all strains harbored bla_{SHV} and bla_{TEM} genes, respectively. Similar results in Turkey showed that 74.3% of *E. coli* and *K. pneumoniae* strains had bla_{SHV-1} and also 52.7% of them have bla_{TEM-1} (17). Due to recent studies that have been conducted all around the world, the bla_{TEM} mutation in *K. pneumoniae* is related to each statistical study population, for example in the study of Akpaka *et al*. in 2010, 84.3% of isolates had the bla_{TEM} gene (13). In Bali *et al*. study in Turkey (2010), the prevalence of bla_{TEM} gene was reported 74.4% similar to Pornour *et al*. in 2010 (18, 19). Whereas, the conducted researches in 2007 and 2009 on *K. pneumoniae* strains showed the prevalence of bla_{TEM} gene was 80% and 25%, respectively (20, 21). Another similar study in Shiraz during 2009-2010 indicated that 60% of isolates were ESBL-producers. The bla_{TEM} was reported in 38.3%, both bla_{CTX-M} and bla_{TEM} in 39%, and 13% harbored bla_{TEM}, bla_{SHV} and bla_{CTX-M} (5). Ghafourian *et al*. reported that among 67 isolated samples of *K. pneumoniae* producing ESBLs, 94% were positive for bla_{SHV}, 16.4% contained bla_{TEM}, and 23.9% harbored bla_{CTX-M}. On the other hand, 20.9% of isolates had both bla_{CTX-M} and bla_{SHV} genes, 13.4% carried both bla_{SHV} and bla_{TEM}, and 6% were positive for bla_{TEM} and bla_{CTX-M} (22). The results of this study indicated an increase in the resistance of *K. pneumoniae* strains to the trimethoprim/sulfamethoxazole and cef-

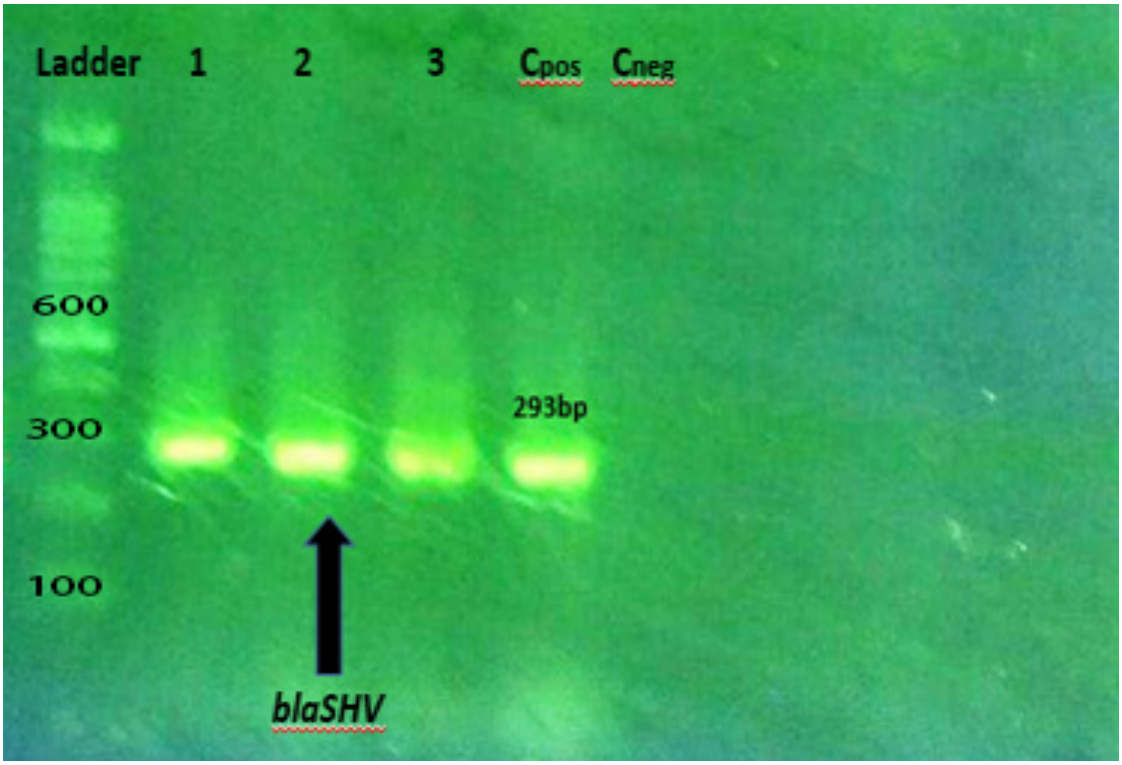

Figure 2. The result of PCR for *bla*$_{SHV}$ C-pos: Positive control, C-neg: Negative control, Left line: 100bp DNA ladder, Line 1, 2 and 3: Positive isolates (293bp).

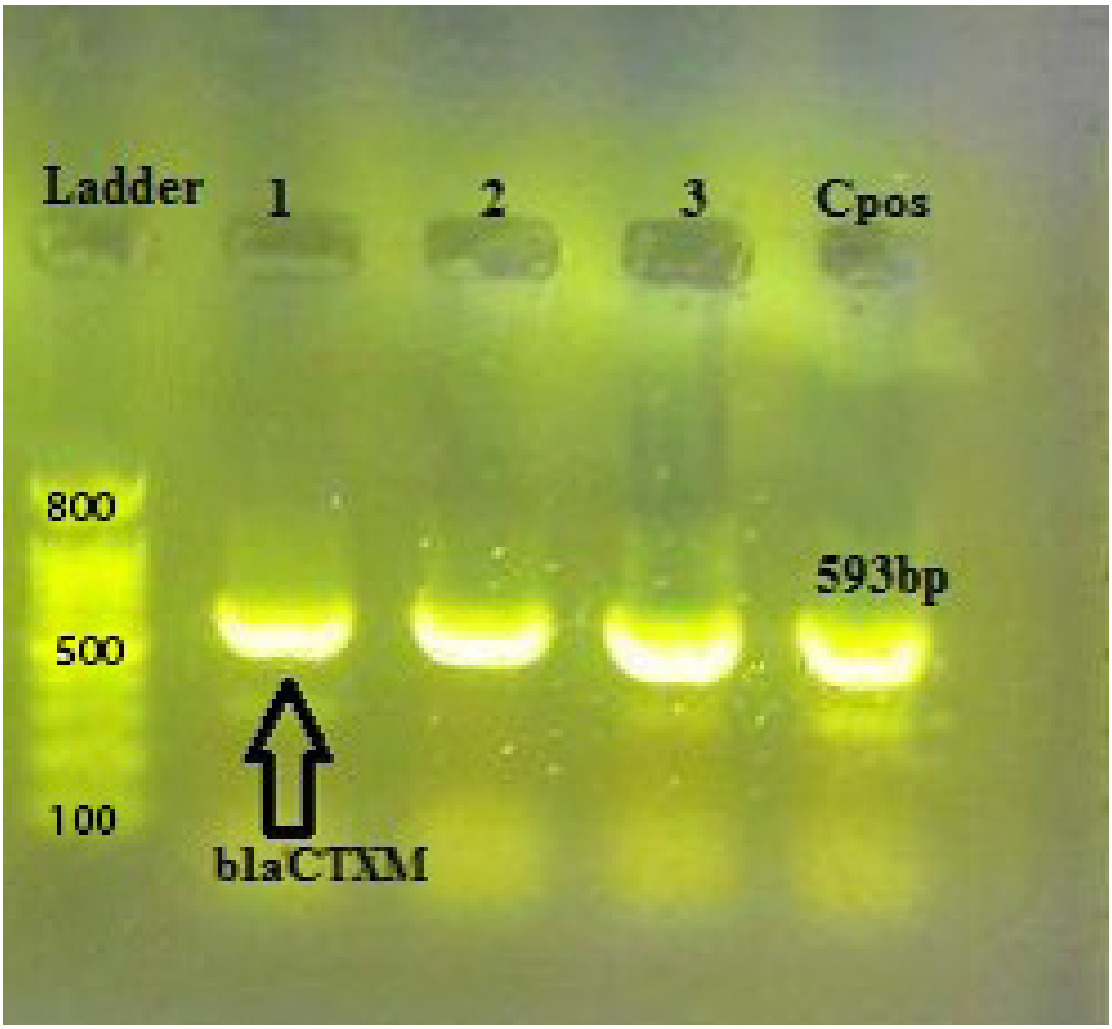

Figure 3. The result of PCR for *bla*$_{CTX-M}$ C-pos: Positive control, Left line: 100bp DNA ladder, Line 1, 2 and 3: Positive isolates (593bp).

Table 2. The frequency of susceptibility and resistant antibiotics among isolated *K. pneumoniae* strains

Antibiotics	Resistant N (%)	Sensitive N (%)	Intermediate N (%)
Ceftazidime	30 (60%)	20 (40%)	-
Cefotaxime	29 (58%)	19 (38%)	2 (4%)
Ciprofloxacin	20 (40%)	29 (58%)	1 (2%)
Ceftriaxone	16 (32%)	32 (64%)	2 (4%)
Co-trimoxazole	19 (38%)	30 (60%)	1 (2%)
Imipenem	-	50 (100%)	-
Aztreonam	22 (44%)	26 (52%)	2 (4%)
Amikacin	18 (36%)	31 (62%)	1 (2%)
Nitrofurantoin	12 (24%)	36 (72%)	2 (4%)
Cefepime	13 (26%)	35 (70%)	2 (4%)
Cefixime	15 (30%)	34 (68%)	1 (2%)
Piperacillin	26 (52%)	22 (44%)	2 (4%)

Table 3. The distribution of MDR among isolated K. *pneumoniae* strains

Antibiotic Name	N (%)
CAZ-CTX-CIPR-CRO-AMI-AZT-CO-FEP-CFM-PIPRA	3 (6%)
CAZ-CTX-CIPR-CO-AZT-AMI-FM-FEP-PIPRA	4 (8%)
CTX-CIPR-CRO-CO-AZT-AMI-CFM-FEP-CAZ	2 (4%)
CAZ-FM-CTX-CIPR-CFM-CO-AMI-PIPRA	4 (8%)
CAZ-CTX-CIPR-FM-AZT-CFM-PIPRA-CRO	2 (4%)
CAZ-CTX-CIPR-FM-AZT-CFM-AMI	1 (2%)
CTX-CFM-CO-CAZ-CRO-PIPRA	2 (4%)
CTX-CIPR-AZT-AMI-FEP-CAZ	1 (2%)
CTX-CIPR-CO-AZT-FEP-AMI	3 (6%)
CAZ-CTX-CRO-AZT-PIPRA	4 (8%)
CRO-AZT-FM-PIPRA-CIPR	1 (2%)
CAZ-CTX-PIPRA-CRO	4 (8%)
CAZ-CTX-PIPRA-CFM	2 (4%)
CAZ-CTX-AZT-CO	1 (2%)

Ceftazidime-Cefotaxime (CTX) -Ciprofloxacin (CP) -Ceftriaxone (CRO) -Aztereonam (AZT) -Amikacin (AN)
Nitrofurantoin-Cefepime (FEP)-Cefaxime (CFM)-Piperacillin (PIPRA) - Co-trimoxazole (CO)

triaxone antibiotics. In conclusion, preventing and also controlling MDR strains is considered as an important issue in the treatment of infections. On the other hand, the insufficient information about the frequency of these genes and genetic patterns can be led to the increase of antibiotic resistance prototype in Iran. Therefore, the detection of *K. pneumoniae* strains containing beta-lactamase resistance enzymes is important for better treatment and preventing the outbreak of these genes.

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Conflict of interest

The authors declare that there is no conflict of interest.

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