International Journal of Advanced Biological and Biomedical Research
Available online at http:www.ijabbr.com
Volume 8, Issue 1 (2020) pp. 75-85 DOI: 10.33945/SAMI/IJABBR.2020.1.8

Original Article

Epidemiological and Genetic Overview of the *Klebsiella pneumoniae* Carbapenemases (KPCs) in *K. pneumoniae* Isolated from the Clinical Samples in Iran

Narjes Mohammadi Bandari[1], Hossein Keyvani[2*], Mohsen Zargar[1], Malihe Talebi[3], Mohammad Reza Zolfaghari[1]

[1]Department of Biology, Qom Branch, Islamic Azad University, Qom, Iran
[2]Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]Department of Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

*Corresponding Author E-mail: Keyvanlab@yahoo.com

Received: 12 June 2019, Revised: 20 August 2019, Accepted: 30 August 2019

ABSTRACT

Background: The prevalence of carbapenem-resistant *Enterobacteriaceae*, especially *Klebsiella pneumoniae* carbapenemase (KPC), has been recently reported worldwide. Therefore, there is an indispensable need for precise and rapid detection of these carbapenemases.
Objectives: This study was aimed to propose an accurate and rapid method for detecting *K. pneumoniae* carbapenemase genes from clinical samples, using reverse transcription-polymerase chain reaction (RT-PCR) and to evaluate the expression of these genes in the presence of *β*-lactam antibiotic by real-time PCR assay.
Methods: One hundred and eighty-one *K. pneumoniae* strains were collected from patients presenting to Firoozgar Hospital of Tehran, Iran. The strains were tested using the disk diffusion method, the modified Hodge test (MHT), and E-test minimum inhibitory concentration (MIC). Next, reverse transcription-PCR method was applied for the identification of *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ genes. Finally, expression of genes was measured by real-time PCR assay in the presence and absence of *β*-lactam antibiotic.
Results:Phenotypic testing showed a high level of antibiotic resistance, while the genotypic methods indicated the presence and expression of carbapenemase genes.
Conclusions: The findings suggest revisions in the current antibiotic therapy protocols, considering the high expression level of resistant carbapenemases to *K. pneumoniae* strains.

Key words: *Bla* $_{OXA-23}$, *Bla* $_{OXA-48}$, E-test, MHT, Real-time PCR.

Introduction

The World Health Organization (WHO) has recognized the antimicrobial resistance (AMR) as a major global health problem in recent decades (Prestinaci *et al.*, 2015). AMR has also led to

elevated mortality rates of nosocomial infections (Kritsotakis *et al.*, 2017). The extensive dispersion of multidrug-resistant phenotypes of carbapenem-resistant Enterobacteriaceae compromises the control of healthcare-associated infections (Kelly *et al.*, 2017). Genes causing AMR in bacteria are located on plasmids of various sizes. They can be transferred from one bacterium to another, from one person to another, and from one country to another (Zacharczuk *et al.*, 2014). *K. pneumonia* is a Gram-negative bacillus, belongs to the Enterobacteriaceae family. It is an opportunistic pathogen, causing human nosocomial infections, septicemia, pneumonia, urinary tract infections, meningitis, diarrhea, and soft tissue infections (Mosavian *et al.*, 2016). The rising multi-drug resistance (MDR) of *K. pneumoniae* isolates has led to limited treatment options for antibiotic therapy and infection control (García-Sureda *et al.*, 2011). Changes in *K. pneumoniae* enzymes have made them resistant to antibiotics such as penicillins, cephalosporins, carbapenems, aminoglycosides, macrolides, and sulfamethoxazole (Doern *et al.*, 2011). Among different metabolic processes, *β*-lactamase is recognized as the main survival and defense mechanism of these pathogenic bacteria against *β*-lactam antibiotics (Drawz and Bonomo, 2010). Carbapenemase-producing Enterobacteriaceae (CPE) in hospitalized patients has been a major concern for more than a decade (Tzouvelekis *et al.*, 2014). Carbapenems comprise a class of beta-lactam antibiotics with significant antimicrobial activities (Nordmann *et al.*, 2012). Carbapenemases can hydrolyze all ß-lactams, including imipenem, ertapenem, meropenem, and doripenem (Lee *et al.*, 2016). Evidence suggests that misuse and overuse of carbapenems are correlated with the increasing antibiotic resistance of *K. pneumoniae* carbapenemase (KPC)-producing bacteria (Haji Hashemi *et al.*, 2016). Generally, carbapenemase enzymes, identified in Enterobacteriaceae, belong to one of three classes of *β*-lactamase, i.e., class A, B, or D. (Bush and Jacoby, 2010). *β*-lactamase class A is becoming more prevalent in the Enterobacteriaceae family, with activities against carbapenems. Class D carbapenemases can be phylogenetically divided into different enzymes, such as *bla* $_{\text{OXA-23}}$ and *bla* $_{\text{OXA-48}}$ (Vasoo *et al.*, 2013). These enzymes have been detected in *K. pneumoniae* strains, isolated from hospitalized patients. Clinical evidence has confirmed their potential to cause antibiotic resistance by hydrolyzing *β*-lactam antibiotics, including monobactams, carbapenems, and third-generation cephalosporins (Lee and Lee, 2010). Early and rapid detection of KPC-producing pathogens is essential for limiting the serious consequences of potential hospital infections by adopting proper infection control measures. This study proposes a rapid detection technique for identifying carbapenem resistance in hospitalized patients by examining the presence of carbapenemases in *K. pneumoniae* as a highly common bacterium. In this study, the emergence of *Klebsiella* species, with carbapenem-hydrolyzing class D *β*-lactamase genes (*bla* $_{\text{OXA-23}}$ and *bla* $_{\text{OXA-48}}$), was reported in the clinical samples. Finally, the expression of genes in the presence and absence of *β*-lactam antibiotic was measured by real-time polymerase chain reaction (PCR) assay.

Materials and methods

Study period and sample collection

This cross-sectional study was conducted in Firoozgar Hospital, Tehran, Iran. Samples collected between March 2018 and December 2018. For this purpose, the patients were admitted to relevant wards. One hundred and eighty-one *K. pneumoniae* clinical samples were collected from the blood, stool, sputum, urine, burn wound, cerebrospinal fluid, trachea, skin lesion, eye discharge, abscess, and catheter.

Bacterial isolation

The *K. pneumoniae* colonies were identified *via* Gram staining, as well as standard biochemical tests including indole, motility, citrate, urease, lactose fermentation, lysine decarboxylase, and MR-VP.

Antimicrobial susceptibility testing: disk diffusion method

Antibiotic discs were used to perform susceptibility tests via the disk diffusion method, comprised of ertapenem (10 µg), imipenem (10 µg), meropenem (10 µg), doripenem (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), piperacillin (100 µg), and aztreonam (30 µg) based on the CLSI guidelines on the Mueller-Hinton agar plate. In the disk susceptibility test, *K. pneumoniae ATCC 13883* strain was a positive control.

Detection of the carbapenemase production: modified hodge test

Carbapenem-resistant strains were exposed to the Modified Hodge Test (MHT) for the detection of carbapenemases production according to CLSI guidelines, 2016 (CLSI, 2016). After preparing a standard suspension of carbapenem-sensitive *Escherichia coli* (0.5 McFarland turbidity) in sterile saline, the solution was diluted in sterile saline (1:10). Then, using the standard disk diffusion method, it was inoculated on a Mueller-Hinton agar plate. The plate was left to dry for five minutes, and then, an ertapenem disk was placed at its center. Three to five colonies were picked from the organism using a swab and inoculated from the disk edge up to at least a 20-mm distance.

After incubating the plates overnight at 37 ^{o}C, they were evaluated on the following day. They were then examined for improved growth around the organism streak at the intersection of the inhibition zone and the streak. The increased *E. coli* growth was indicative of carbapenemase formation by the tested organism, inactivating ertapenem. Also, an indentation was detected in the zone considering the presence of a cloverleaf-shaped or distorted inhibition zone. The CLSI guidelines interpret this phenomenon as positive in favor of carbapenemase production in the isolates.

Minimum inhibitory concentration (MIC): epsilometery test

The last test of resistance to carbapenems was an Epsilometery test intended for obtaining the minimum inhibitory concentration (MIC) in an imipenem disk. To this end, initially, a concentration of 0.5 McFarland standard was prepared from that MHT-positive isolates. Afterward, a sterile cotton swab was dipped into and pulled out slowly from the inoculums. The swab was rotated several times against the inner wall of the tube above the fluid level to removed excess liquid on Muller-Hinton agar plate. E-test strips (bioMérieux) were placed on the plate with "E"-end at the periphery of the plate. After incubating the plates for 24 hours at 37 °C, the MIC was read and recorded at the point where ellipse met the scale.

Molecular identification of bla $_{OXA-23}$ and bla $_{OXA-48}$ genes in K. pneumoniae by reverse transcription-polymerase chain reaction (RT-PCR)

The RT-PCR technique was applied to evaluate isolates with positive results on the modified Hodge test (MHT) for detection of *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ genes. RT-PCR was performed using specific primers for *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ to prevent false-positive results. Specific primer pairs,

targeting *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ genes, were designed by IDT software and synthesized by Macrogen Company (Korea) (Table 1).

Table 1. Primer sequences of studied virulence genes in *K. pneumoniae* isolates and reaction setup for 2-step real-time PCR

Primer Sequences	RT-PCR(step1)		Real-Time PCR(step2)	
	Conditions	Volume Re-actions	Conditions	Volume Re-actions
bla $_{OXA-23}$ F-GACACTAGGAGAAGCCATCAAG R-TGCATGAGATCAAGACCGATACT *bla* $_{OXA-48}$ F-ACATAAATCACAGGGCGTAGTT R- CGAGGGCGATCAAGCTATT *rpo B* F-AACCCGCTGTCTGAGATTAC R-GGCGTTTCGATCGGACATA	1cycle 25ċ.....10min 47ċ.....60min 70ċ.....10min	Total RNA 5µl Random hexamer 2µl H_2O up to 10µl	1cycle 95ċ.....10min 40cycle: 95ċ.....30s 58ċ.....30s 72ċ.....45s 1cycle: 95ċ.....30m	*Real Q Plus 2x Master Mix, green*: 12.5 µl Primer F+R Primer: 2 µl Template DNA: 5 µl Nuclease-free water: 5.5µl Total reaction volume:25 µl

Isolation of total RNA

After inoculating the strains from glycerol stocks in nutrient agar (2 mL), they were grown at 37ºC overnight. Then, the strains were sub-cultured in the nutrient medium (5 mL) and grown to the mid-exponential phase (OD600=1.5–2.0). Afterward, an aliquot of the culture (0.25 mL) was added to 2 mL of RNX-plus (Sinaclon, Iran). In addition, isolation of total RNA was performed based on the manufacturer's guidelines. *DNase* treatment was applied to remove the residual DNA with 20 U of RQ1 *DNase I*. (Sinaclon, Iran).

cDNA synthesis

A reaction mixture, consisting of 5 µg of RNA, was incubated in DEPC-treated water and 1 µL of random hexamers (Pars Tous, Iran). Then, it was incubated for five minutes at 65 ºC and cooled down on the ice. After 10 µL of RT premixture (2X) was added, it was mixed by pipetting up and down gently in a total volume of 24 µL. Incubation was then performed at 25 ºC for 10 minutes and at 47 ºC for 60 minutes. Following that, the reaction was terminated by heating at 70 ºC for 10 minutes and chilling on ice. The collected cDNA was stored at -20 ºC until further analysis.

Real-time PCR assay

Changes in *bla* gene expression were measured, using RT-PCR in the presence and absence of imipenem, in 36 antibiotic-resistant clinical strains containing two genes. Quantitative PCR (qPCR) was also performed according to the manufacturer's instructions. SYBR Green and 2X RT-PCR Master Mix Green were used in a No-Rox kit, and then, an RT-PCR detection system (Corbett, Australia) was used. Based on the melt curve analysis after 40 cycles, the presence of RT-PCR product was examined. The reference housekeeping gene was *rpoB* gene. The Rest software was used to calculate the gene expression, design of the graphs and the critical threshold cycle (CT). Then, the expression analysis was performed by relative measurement of

mRNA expression in comparison with *K.pneumoniae ATCC 13883* strain. That way, the comparative Ct (ΔΔCt) method was applied to determine Ct values; fold differences were measured as $2^{-\Delta\Delta Ct}$.

Statistical analysis

To analyze the obtained data, SPSS version 23.0 (SPSS, Chicago, IL) using t-test was employed; for data analysis were performed of descriptive statistics (frequency, percentage, mean); the level of significance in the current study was <0.05.

Results

One hundred and eighty-one *K. pneumoniae* strains were collected from clinical samples such as aspirate (n=65), sputum (n=40), urine (n=35), blood (n=26) and other clinical samples (n=15). Cefepime and imipenem showed the highest and lowest rates of resistance, respectively. *K. pneumoniae* exhibited the highest susceptibility to meropenem (38.12%) and the lowest susceptibility to cefepime (14.36%). Therefore, all the samples were confirmed as multidrug-resistant (MDR) strains.

One-hundred strains, such as aspirate, sputum, urine, blood, and other clinical samples, were positive as KPC-producing in MHT (clover leaf-shaped) (Figure 1). The results of standard minimum inhibitory concentration (MIC) test were in agreement with those of MHT. The MICs ranged from 4 µg/mL to 6 µg/mL (MIC ≥ 4 µg/mL). Therefore, these strains exhibited resistance to imipenem (Figure 2).

The findings showed that 51% (n= 51) of strains were *bla* $_{OXA\ -23}$ positive, and 84% (n= 89) were *bla* $_{OXA\ -48}$ positive; also, 92% (n=92) of the strains carried both genes (Table 2 and Figure 3).

Moreover, the gene expression of *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ was examined in this study. We observed the overexpression of these genes when induced by 2 mg. L^{-1} of imipenem. The results demonstrated that imipenem caused 1.27 and 20.53-fold increase in the expression of genes, respectively.

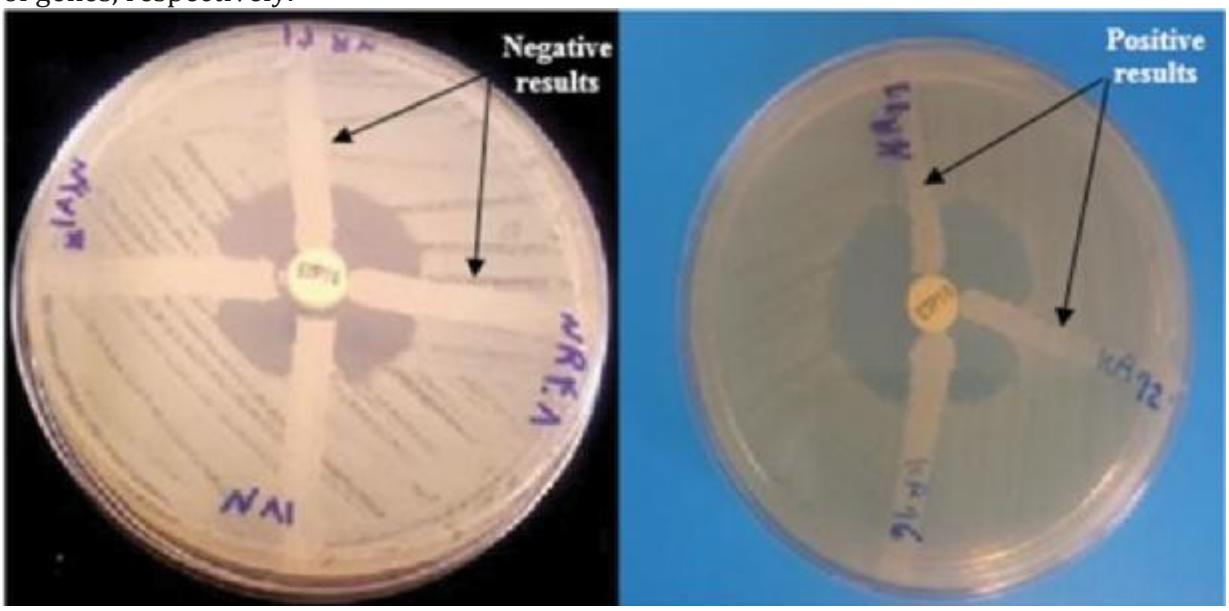

Figure 1. MHT test modified hodge test (MHT) showing positive and negative results

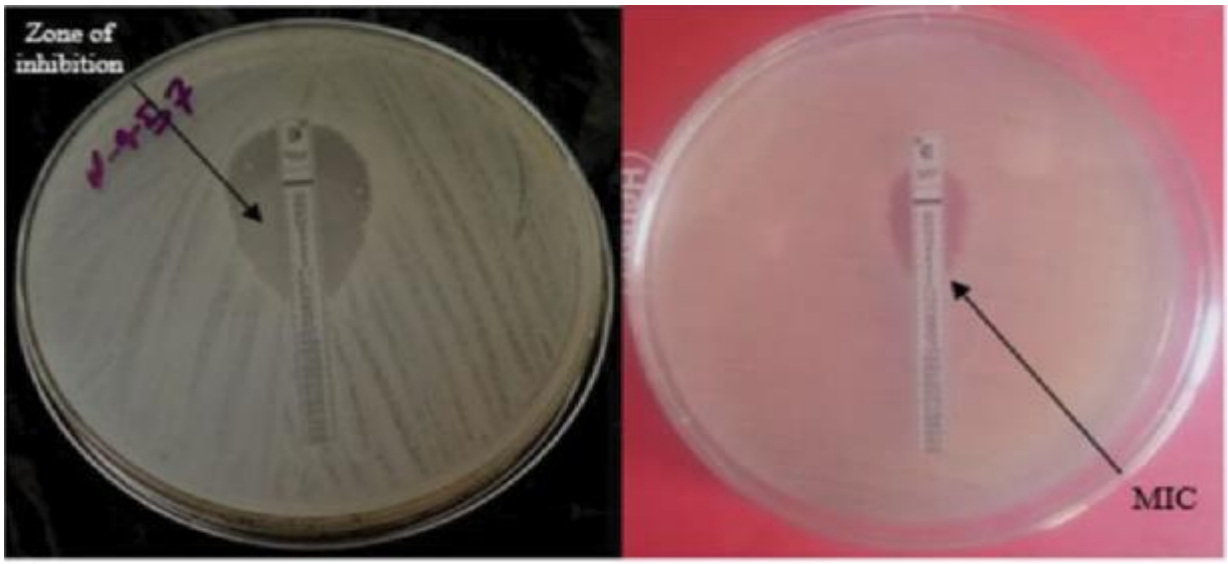

Figure 2. Epsilometery Test E-test inhibition ellipse showing the MIC at the intersection of the growth of the organism

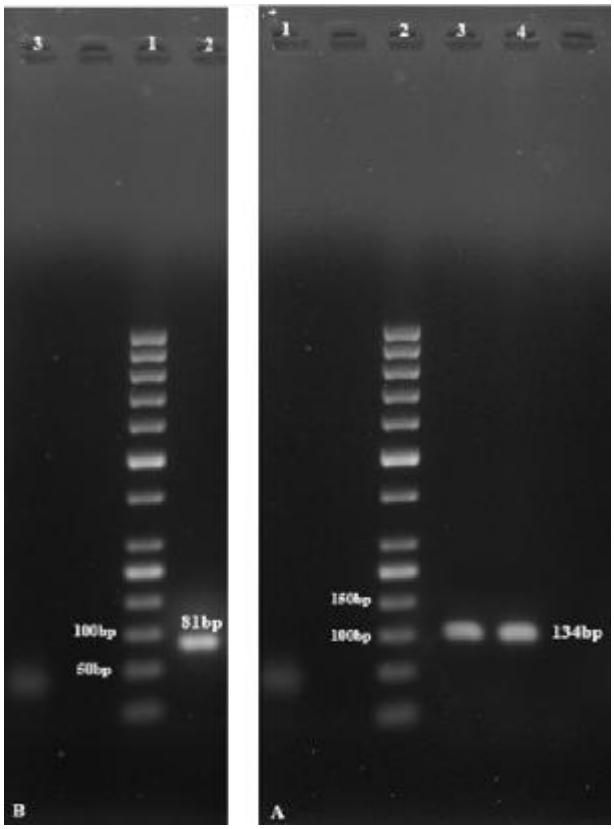

Figure 3. Agarose gel electrophoresis of PCR products

A. Gel electrophoresis shows the PCR product of *bla* $_{OXA-48}$: Lane 1. Ctrl-, Lane 2. Gene ruler 25 bp DNA Ladder, Lane 3. Ctrl+ (*K. pneumoniae ATCC 13883* strain), Lane 4. PCR product of *bla* $_{OXA-48}$, B. Gel electrophoresis shows the PCR product of *bla* $_{OXA-23}$: Lane 1. Gene ruler 25 bp DNA Ladder, Lane 2. PCR product of *bla* $_{OXA-23}$, Lane 3. Ctrl-

Table 2. Antimicrobial resistance profile of *K. pneumoniae* isolates

Sensitive(S)	Intermediate (I)	Resistant(R)	Antibiotic Name
66 (36.5%)	15 (8.28%)	100 (55.2%)	Imipenem
69 (38. 2%)	10 (5.24%)	102 (56.3%)	Meropenem
65 (36%)	14 (7.7%)	102 (56.3%)	Ertapenem
66 (37%)	10 (5.24%)	105 (58%)	Doripenem
29 (16.02%)	2 (1.1%)	150 (83.5%)	Cefotaxime
31 (17.12%)	5 (2.76%)	145 (80.1%)	Ceftazidime
26 (14.36%)	3 (1.65%)	152 (83.9%)	Cefepime
46 (25.41%)	14 (7.73%)	121 (66.8%)	Cefoxitin
29 (16.02%)	4 (2.2%)	148 (81.7%)	Ceftriaxone
28 (15.47%)	3 (1.65%)	150 (82.7%)	Gentamicin
29 (16.02%)	4 (2.2%)	148 (81.7%)	Piperacillin
60 (33.14%)	13 (7.18%)	108 (59.6%)	Aztreonam

Discussion

Nosocomial pathogens are important carbapenemase-producing *K. pneumoniae* strains. The prevalence of KPC-producing *K. pneumoniae* in hospitals is associated with increased mortality (Tzouvelekis *et al.*, 2014). In addition to KPC-type carbapenemases, OXA-type enzymes are also found in *K. pneumoniae*. Currently, isolation and identification of *bla* $_{oxa}$ genes are a challenge for diagnostic laboratories. The presence of *bla* $_{oxa}$ merits special consideration, as the plasmid containing this gene leads to MDR and can be an epidemiological challenge, limiting the therapeutic options and increasing the mortality rate (Vasoo *et al.*, 2013). Carbapenems are often applied as first-line treatment for drug-resistant Gram-negative organisms, while the increasing frequency of KPC organisms has decreased the efficacy of these antibiotics (Codjoe and Donkor, 2018). In this study, the lowest resistance was attributed to carbapenems based on the antibiotic susceptibility tests; this finding is consistent with the results of a study by Bina *et al.*, which indicated the highest resistance to carbapenem (Bina *et al.*, 2015). Considering the increasing spread of antimicrobial resistance (AMR) in hospital settings, the findings of the present study are similar to those reported by (Eilertson *et al.*, 2017), and (Kontopoulou *et al.*, 2010). These findings warrant more attention by the intensive care unit personnel to limit the potential risk of AMR in hospitals.

Our study showed that the resistant isolates to imipenem by E-test were 100 out of 181 (55.2%), which is in agreement with the results of Al Hindi and El Shalakany (2016). On the other hand, in a study conducted by Gupta *et al.*, in New York, USA, carbapenem resistance was reported by E-test in 10.8% of the isolates (Gupta *et al.*, 2011). In the present study, we found that the strains were positive for KPC-producing organisms, based on MHT, which is in agreement with the results of (Cury *et al.*, 2012; Fattouh *et al.*, 2015; Pasteran *et al.*, 2010; Carvalhaes *et al.*, 2009), as they found MHT (Cloverleaf test) to be a sensitive screening test for the detection of class A carbapenemases and discrimination of false-positive results for *K. pneumoniae*.

The current study was performed for the first time in Iran using RT-PCR technique to determine the pattern of MDR and to investigate *bla* $_{oxa-23}$ and *bla* $_{oxa-48}$ genes in resistant strains of *K. pneumoniae* according to phenotypic tests, as well as in vitro susceptibility tests.

We showed that 84% of *K. pneumoniae* isolates which were resistant to carbapenem were positive for *bla* $_{oxa-23}$ gene and 92% isolates carrying *bla* $_{OXA-48}$, which is in line with previous studies from Iraq Abbas and Jarallah confirmed the presence of *bla* $_{oxa-23}$ gene based on the PCR assay (Abbas and Jarallah, 2017). This is the first outbreak of carbapenem-resistant *K.pneumoniae* isolates producing of *bla* $_{OXA-23}$ is reported in Iran and the first report of *K.pneumoniae* isolates producing of *bla* $_{OXA-48}$ in our hospital.

The first cases of OXA-48-producing *K. pneumoniae* strains in Iran reported by (Azimi *et al*., 2014). In Iran, there is another report of OXA-48 in *K. pneumoniae* by Solgi *et al*., according to their results, the *bla* $_{OXA-48}$ gene was detected in 96 isolates (Solgi *et al*., 2018). Also, in another research in Iran, *bla* $_{OXA-51}$, *bla* $_{OXA-23}$, *bla* $_{VIM}$, and *bla* $_{KPC}$ genes have been reported in *A. baumannii* (Azimi *et al*., 2015), that is, closest to our findings.

The antimicrobial resistance gene expression of *K. pneumoniae* was measured by real-time PCR assay. We found that the expression rate of *bla* $_{OXA-23}$ and *bla* $_{OXA-48}$ in carbapenem-resistant *K.pneumoniae* isolates from Firoozgar hospital is undoubtedly high.

Farivar *et al*., evaluated the expression level of oqxA and acrA genes among *K. pneumoniae* isolated. Real-time PCR assay showed a higher expression level of oqxAB (2.3 folds) and acrAB (4 folds) pumps in resistant strains, that is like with the result of our study (Farivar *et al*., 2016).

Also, the results of real-time PCR showed a two-fold increase in the expression of *bla* $_{oxa-23}$ and *bla* $_{oxa-48}$ genes in the presence of 2 mg. L^{-1} of imipenem. These results are consistent with a four-to eight-fold increase in MexX and mexY gene expression of *Pseudomonas aeruginosa* in the presence of tetracycline (2 mg/L) (Dumas *et al*., 2006). Moreover, our results of the gene expression are in agreement with the study by Dhban et al., who showed the overexpression of 12 pilus genes in resistant *Acinetobacter baumannii* isolates by three folds when treated with a sub-MIC of imipenem (Dhabaan *et al*., 2016).

MHT was applied as a suitable method for evaluating the production of carbapenemase in the present study. Also, the RT-PCR method provided a convenient molecular tool for the detection and expression of *bla* $_{oxa-23}$ and *bla* $_{oxa-48}$ genes, providing a precaution for the actual outbreak. To the best of our knowledge, this study is the first report of the expression of *bla* $_{oxa-23}$ and *bla* $_{oxa-48}$ genes from *K. pneumoniae* strains in Iran. We also reported the emergence of an unprecedented oxa variant (oxa-23) in a *K. pneumoniae* isolate. Our findings revealed the high prevalence of oxa-23 and oxa-48 genes, encoding carbapenem resistance among *K. pneumoniae* isolates in Firoozgar Hospital, Tehran, Iran.

Conclusion

Based on the present study and other similar researches, the emergence and spread of KPC-producing, extreme drug-resistant *K. pneumoniae* isolates are increasing in hospital settings. Therefore, it is important to control the release of KPC-producing organisms by making changes in infection control strategies and administering appropriate antibiotic treatments in areas where patients have long hospital stays. Based on the findings, real-time PCR assay can reduce the turnaround time for infection control measures and detection of carbapenemase-producing organisms. Also, the current methods of laboratory diagnosis need to be improved to detect KPC resistance. Overall, the proposed method in this study can be considered an improvement to these methods. We hope that our findings can be applied for the implementation of an effective systematic strategy to manage

infectious diseases and prevent the diffusion and dispersion of KPC-producing *K. pneumoniae* in Iran.

Acknowledgments

The authors would like to thank the staff of the Microbiology Laboratory in Firoozgar Hospital Tehran and Keyvan research lab who contributed to this research

References

Abbas, FM, Jarallah, EM. (2017). Detection of OXA-23 among Carbapenem-Resistant Clinical Isolates of *Klebsiella pneumoniae* in Hilla. *JUBPAS*, 25(2):454-435.

Al Hindi, AA, El Shalakany, AH. (2016). Evaluation of a real-time PCR assay for the detection of the *Klebsiella pneumoniae* carbapenemase gene (*bla*KPC) in Enterobacteriaceae isolates from clinical samples in Menoufia University Hospitals. *Egypt Afr. J. Microbiol.*, 10:1397-1407.

Azimi, L, Nordmann, P, Lari, AR, Bonnin, RA. (2014). First report of OXA-48-producing *Klebsiella pneumoniae* strains in Iran. *GMS Hyg. Infect. Control*, 9(1). doi: 10.3205/dgkh000227.

Azimi, L, Talebi, M, Pourshafie, MR, Owlia, P, Lari, AR. (2015). Characterization of carbapenemases in extensively drug resistance Acinetobacter baumannii in a burn care center in Iran. *Int. J. Mol. Cell Med.*, 4(1):46.

Bina, M, Pournajaf, A, Mirkalantari, S, Talebi, M, Irajian, G. (2015). Detection of the *Klebsiella pneumoniae* carbapenemase (KPC) in *K. pneumoniae* Isolated from the Clinical Samples by the Phenotypic and Genotypic Methods. *Iran J. Pathol.*, 10(3):199.

Bush, K, Jacoby, GA. (2010). Updated functional classification of *β*-lactamases. *Antimicrob Agents Chemother.*, 54(3):969-976.

Carvalhaes, CG, Picão, RC, Nicoletti, AG, Xavier, DE, Gales, AC. (2009). Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false-positive results. *J. Antimicrob. Chemother.*, 65(2):249-251.

Codjoe, F, Donkor, E. (2018). Carbapenem resistance: a review. *Med. Sci.*, 6(1):1.

Cury, AP, Andreazzi, D, Maffucci, M, Caiaffa-Junior, HH, Rossi, F. (2012). The modified Hodge test is a useful tool for ruling out *Klebsiella pneumoniae* carbapenemase. *Clinics*, 67(12):1427-1431.

Dhabaan, GN, AbuBakar, S, Cerqueira, GM, Al-Haroni, M, Pang, SP, Hassan, H. (2016). Imipenem treatment induces expression of important genes and phenotypes in a resistant Acinetobacter baumannii isolate. *Antimicrob Agents Chemother.*, 60(3):1370-1376.

Doern, CD, Dunne, WM, Burnham, CAD. (2011). Detection of *Klebsiella pneumoniae* carbapenemase (KPC) production in non-*Klebsiella pneumoniae* Enterobacteriaceae isolates by use of the Phoenix, Vitek 2, and disk diffusion methods. *J. Antimicrob Chemother.*, 49(3), 1143-1147.

Drawz, SM, Bonomo, RA. (2010). Three decades of β-lactamase inhibitors. *Clin. Microbiol.*, 23(1):160-201.

Dumas, JL, Van Delden, C, Perron, K, Köhler, T. (2006). Analysis of antibiotic resistance gene expression in Pseudomonas aeruginosa by quantitative real-time-PCR. *FEMS Microbiol. Lett.*, 254(2):217-225.

Eilertson, B, Chen, L, Chavda, KD, Kreiswirth, BN. (2017). Genomic characterization of two KPC-producing Klebsiella isolates collected in 1997 in New York City. *Antimicrob Agents Chemother.*, 61(4):e02458-02416.

Fattouh, M, El-Din, AN, Omar, MA. (2015). Detection of *Klebsiella pneumoniae* carbapenemase (KPC) producing gram-negative superbugs: an emerging cause of multidrug-resistant infections in General Surgery Department of Sohag University Hospital, Egypt. *Int. J. Curr Microbiol. App. Sci.*, 4(5):1-15.

García-Sureda, L, Juan, C, Doménech-Sánchez, A, Albertí, S. (2011). Role of *Klebsiella pneumoniae* LamB Porin in antimicrobial resistance. *Antimicrob Agents Chemother.*, 55(4):1803-1805.

Guo, Y, Cen, Z, Zou, Y, Fang, X, Li, T, Wang, J, Chang, D, Su, L, Liu, Y, Chen, Y, Yang, R, Liu, C. (2012). Whole-genome sequence of Klebsiella pneumonia strain LCT-KP214. *J. Bacteriol*, 194:3281-3281.

Gupta, N, Limbago, BM, Patel, JB, Kallen, AJ. (2011). Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin. Infect Dis.*, 53(1):60-67.

Haji Hashemi, B, Farzanehkhah, M, Dolatyar, A, Imani, M, Farzami, M, Rahbar, M, Hajia, M. (2012). A study on prevalence of KPC-producing from *Klebsiella pneumoniae* using Modified Hodge Test and CHROMagar in Iran. *Ann. Biol. Res.*, 3(12):5659-5664.

Kelly, AM, Mathema, B, Larson, EL. (2017). Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. *Int. J. Antimicrob. Agents*, 50(2):127-134.

Kontopoulou, K, Protonotariou, E, Vasilakos, K, Kriti, M, Koteli, A, Antoniadou, E, Sofianou, D. (2010). Hospital outbreak caused by *Klebsiella pneumoniae* producing KPC-2 *β*-lactamase resistant to colistin. *J. Hosp. Infec.*, 76(1):70-73.

Farivar, AS, Nowroozi, J, Eslami, G, Sabokbar, A, Hashemi, A. (2016). The study of antibiotic resistance among *Klebsiella pneumoniae* and expression level of oqxA and acrA genes by using real-time PCR. *Res. Med.*, 40(1):42-48.

Kritsotakis, EI, Flora Kontopidou, EA, Roumbelaki, M, Ioannidou, E, Gikas, A. (2017). Prevalence, incidence burden, and clinical impact of healthcare-associated infections and antimicrobial resistance: a national prevalent cohort study in acute care hospitals in Greece. *Infect. Drug Resist.*, 10:317.

Lee, CR, Lee, JH, Park, KS, Kim, YB, Jeong, BC, Lee, SH. (2016). Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. *Front. Microbiol.*, 7:895.

Lee, JH, Lee, SH. (2010). Carbapenem resistance in gram-negative pathogens: Emerging non-metallo-carbapenemases. *Res. J. Microbiol.*, 5(7):607-628.

Nordmann, P, Poirel, L, Dortet, L. (2012). Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg. Infect. Diseases*, 18(9):1503.

Pasteran, F, Mendez, T, Rapoport, M, Guerriero, L, Corso, A. (2010). Controlling false-positive results obtained with the Hodge and Masuda assays for detection of class A carbapenemase in species of Enterobacteriaceae by incorporating boronic acid. *J. Clin. Microbiol.*, 48(4):1323-1332.

Prestinaci, F, Pezzotti, P, Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health*, 109(7):309-318.

Solgi, H, Badmasti, F, Giske, CG, Aghamohammad, S, Shahcheraghi, F. (2018). Molecular epidemiology of NDM-1-and OXA-48-producing *Klebsiella pneumoniae* in an Iranian hospital: clonal dissemination of ST11 and ST893. *J. Antimicrob Chemother.*, 73(6):1517-1524.

Tzouvelekis, L, Markogiannakis, A, Piperaki, E, Souli, M, Daikos, G. (2014). Treating infections caused by carbapenemase-producing Enterobacteriaceae. *Clin. Microbiol. Infect.*, 20(9):862-872.

Vasoo, S, Cunningham, SA, Kohner, PC, Simner, PJ, Mandrekar, JN, Lolans, K, Patel, R. (2013). Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J. Clin. Microbiol.*, 51(9):3097-3101.

Zacharczuk, K, Piekarska, K, Szych, J, Zawidzka, E, Sulikowska, A, Wardak, S, Jagielski, M, Gierczyński, R. (2011). Emergence of *Klebsiella pneumoniae* coproducing KPC-2 and 16S rRNA methylase ArmA in Poland. *Antimicrob. Agents Chemother.*, 55(1):443-446.

How to cite this article: Narjes Mohammadi Bandari, Hossein Keyvani, Mohsen Zargar, Malihe Talebi, Mohammad Reza Zolfaghari, Epidemiological and Genetic Overview of the Klebsiella pneumoniae Carbapenemases (KPCs) in K. pneumoniae Isolated from the Clinical Samples in Iran. *International Journal of Advanced Biological and Biomedical Research*, 2020, 8(1), 75-85. Link: http://www.ijabbr.com/article_36267.html