

Prevalence of Acquired Carbapenemase Genes in *Klebsiella Pneumoniae* by Multiplex PCR in Isfahan

Abstract

Background: Multi-drug resistant *Klebsiella pneumoniae* has been considered as a serious global threat. This study was done to investigate carbapenemase producing genomes among *K. pneumoniae* isolates in Isfahan, Central Iran. **Materials and Methods:** In a cross-sectional study from 2011 to 2012, 29 carbapenem resistant (according to disc diffusion method) carbapenemase producing (according to modified Hodge test) *K. pneumoniae* strains were collected from Intensive Care Unit (ICUs) of Al-Zahra referral Hospital. In the strains with the lack of sensitivity to one or several carbapenems, beta-lactams, or beta-lactamases, there has been performed modified Hodge test to investigate carbapenemase and then only strains producing carbapenemases were selected for molecular methods. **Results:** In this study, there have been 29 cases of *K. pneumoniae* isolated from hospitalized patients in the (ICU). Three cases (10.3%) contained blaVIM, 1 case (3.4%) contained blaIMP, and 1 case (3.4%) contained blaOXA. The genes blaNDM and blaKPC were not detected. Then, 16 cases (55.2%) from positive cases of *K. pneumoniae* were related to the chip, 4 cases (13.8%) to catheter, 6 cases (20.7%) to urine, and 3 cases (10.3%) to wound. **Conclusion:** It is necessary to monitor the epidemiologic changes of these carbapenemase genes in *K. pneumoniae* in our Hospital. More attention should be paid to nosocomial infection control measures. Other carbapenemase producing genes should be investigated.

Keywords: Carbapenemase, imipenemas, *Klebsiella pneumoniae*, *Klebsiella pneumoniae* carbapenemase, New Delhi metslb-b-lactamase, oxacillinase, Verona integron-encoded metallo-b-lactamase

Farzin Khorvash,
Mohammed Reza
Yazdani,
Ali Asghar Soudi,
Shiva Shabani,
Nirvana Tavahen¹

From the Departments of
Infectious Diseases and
¹Pediatrics, School of Medicine,
Isfahan University of Medical
Sciences, Isfahan, Iran

Introduction

The emergence of carbapenemase producing *Enterobacteriaceae* is of concern because for which, very few (if any) antibiotic alternatives remain available. This is the reason why early detection of carbapenemase producers is significant.^[1] On the other hand, in some cases, despite microbial resistance, there is no increase in minimum inhibitory concentration of carbapenems, and so using molecular techniques and not only phenotypic tests are very helpful to decrease mortality, morbidity, and treatment costs.^[2]

Understanding the background of genetic mechanisms information of resistance to antibiotics can facilitate the ways of prevention, control, and limitation of the infectious diseases. This can be easily performed using polymerase chain reaction (PCR) molecular methods with high reliability. It is necessary to use PCR molecular methods to prove the

conclusive existence of carbapenemase genomes. In fact, PCR molecular methods are the standard techniques to identify carbapenemases.^[3,4]

According to a study of Poirel *et al.*^[3] in 2011, multiplex PCR methods are as suitable as simplex PCR methods to screen productive genes of carbapenemases, and these methods are considered as the rapid and trustworthy methods (<4 h).

In geographical areas that the carbapenemase producing organisms are not endemic, all dominant genes can produce resistance and it is necessary to consider multiplex PCR methods as the first screening method. Also, this method is suitable in epidemiological studies and is an economical method.^[5]

Klebsiella pneumoniae is an opportunistic pathogen from the family *Enterobacteriaceae* capable to cause carbapenem resistant infections in hospitals

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Address for correspondence:
Dr. Mohammed Reza Yazdani,
Department of Infectious
Diseases, School of Medicine,
Isfahan University of Medical
Sciences, Isfahan, Iran.
E-mail: yazdani@med.mui.ac.ir

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especially Intensive Care Units (ICUs). The investigation of the studies has shown that the most common genes producing carbapenemases in *K. pneumoniae* are derived from five genes *K. pneumoniae* carbapenemase (KPC), imipenemase (IMP), Verona integron-encoded metallo- β -lactamase (VIM), oxacillinase (OXA), and New Delhi metallo- β -lactamase (NDM).^[6]

Due to phenotypic methods, there are several reports on the appearance of *K. pneumoniae* resistant to carbapenems obtained from the patients hospitalized in ICUs in Al-Zahra referral Hospital of Isfahan University of Medical Sciences. The purpose of this study is to investigate the prevalence of KPC, IMP, VIM, OXA, and NDM genes in *K. pneumoniae* isolates in Al-Zahra Hospital. Also, due to limited financial resources, there is not possible to investigate other genes in this study.

This study can be helpful in providing initial information on the prevalence of the nosocomial infections by *K. pneumoniae* producing carbapenemases in order to monitor and control the drug resistances and codify suitable policies for the identification and control of the nosocomial infections and planning principles to physicians and health administrations.

Materials and Methods

This is a cross-sectional study, conducted in Al-Zahra referral Hospital, Isfahan in 2011–2012. The protocol was approved by the Medical Ethics Committee of Isfahan University of Medical Sciences.

Twenty-nine carbapenemase producing *K. pneumoniae* specimens were collected from the ICUs of Al-Zahra Hospital of Isfahan University of Medical Sciences. The clinical sources of the specimens included blood, urine, sputum, and wound secretions.

The diagnosis of *Klebsiella* was performed using the colony morphology. Susceptibility profile was identified by Kirby–Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI). The modified Hodge test also was performed to endorse

the existence of carbapenemase enzymes according to CLSI recommendations and only carbapenemases producing strains were selected for molecular methods.

In all samples, DNA was extracted using of phenol-chloroform method and five pair primers blaKPC, blaVIM, blaIMP, blaNDM, and blaOXA with the sizes 232–798 bp and one pair primer blaSHV-1 for the initial control and the confirmation of *K. pneumoniae* (made by Metabion Company, Germany) was purchased in the form of lyophilization [Table 1]. Amplification was performed using thermocycler T-CY (Netherland). Every cycle includes denaturation DNA at 94°C for 10 min and then other amplification includes 30 cycles at 94°C for 20 s, 40 cycles at 52°C for 40 s and 50 cycles at 72°C for 30 s and final extension at 72°C for 5 min. After third stage and final stage of the amplification, the product was stained on an agarose gel (2%), and analyzed with ethidium bromide in voltage 100 for an hour with electrophoresis set (made by Paya Pajooresh, Pars Company, Iran), and then the multiplied genes were separated due to their molecular weight. Finally, the separated genes were observed and recorded the composed images in the detector and recording gel set (UVI doc). Also, detection of genes was compared with positive control.

Results

In this study, there have been 29 cases of *K. pneumoniae* isolated from hospitalized patients in the ICU. The mean ages of the patients were 53.6 ± 18.2 in the range of 20–78 years. Among them, 20 patients were male (69%), and 9 patients were female (31%). The mean age of women and men was 50.4 ± 20.2 and 60.6 ± 10.5 years, respectively, and there was no significant difference between the genders ($P = 0.17$).

As shown in Table 2, the gene expression was shown based on patients age, sex, and source of sample. According to this table, no statistically difference between fore groups based above variables.

The investigation of blaKPC, blaIMP, blaVIM, blaOXA, and blaNDM genes showed that 5 cases (17.2%) from

Table 1: The properties of used primers in PCR reaction

Primer (bp)	Sequence (5'-3')	Gene	Product size
IMP-F	GGAATAGAGTGGCTTAAAYTCTC	blaIMP	232
IMP-R	GGTTTAAAYAAAACAACCACC		
VIM-F	GATGGTGTGTTGGTCGCATA	blaVIM	390
VIM-R	CGAATGCGCAGCACCAG		
OXA-F	GCGTGGTTAAGGATGAACAC	blaOXA	438
OXA-R	CATCAAGTTCAACCCAACCG		
KPC-Fm	CGTCTAGTTCTGCTGTCTTG	blaKPC	798
KPC-Rm	CTTGTCATCCTTGTTAGGCG		232
NDM-F	GGTTTGCGGATCTGGTTTTC	blaNDM	621
NDM-R	CGAATGGCTCATCACGATC		

KPC: *Klebsiella pneumoniae* carbapenemase, IMP: Imipenemase, VIM: Verona integron-encoded metallo- β -lactamase, OXA: Oxacillinase, NDM: New Delhi metallo- β -lactamase, PCR: Polymerase chain reaction

29 cases contain one of these genes. Three cases (10.3%) contained blaVIM, 1 case (3.4%) contained blaIMP, and 1 case (3.4%) contained blaOXA ($P < 0.05$). The genes blaNDM and blaKPC were not detected ($P < 0.05$) [Figure 1].

Then, 16 cases (55.2%) from positive cases of *K. pneumoniae* were related to the chip, 4 cases (13.8%) to catheter, 6 cases (20.7%) to urine, and 3 cases (10.3%) to wound. Due to these results, the greatest resistance was to ampicillin and meropenem compared to imipenem so that each case from 29 cases (100%) were resistant to these antibiotics. Also, 28 samples (6.96%) were resistant to ceftazidime, ciprofloxacin, and cefotaxime and 1 case (3.4%) was susceptible to these three antibiotics. Also, 27 cases (93.1%) were resistant to cefepime, and 23 samples (3.70%) were resistant to amikacin. The only antibiotic affected on these strains effectively was colistin, so that all 29 samples were susceptible to it [Figure 2].

Discussion

The increasing prevalence of multi-drug resistant *K. pneumoniae* isolates has been associated with higher morbidity and mortality rates. The overall purpose of this study is to investigate the prevalence of blaKPC, blaIMP, blaVIM, blaOXA, and blaNDM genes in *K. pneumoniae* by multiplex PCR molecular method in isolated specimens

Table 2: Gene expression base on demographic data

Genes variables	Negative	VIM	IMP	OXA	P
Mean of age (year)	55.9±17.2	53±20.7	25	28	0.19
Sex, n (%)					
Male	17 (70.8)	1 (33.3)	1 (100)	1 (100)	0.65
Female	7 (29.2)	2 (66.7)	0 (0)	0 (0)	
Source of samples, n (%)					
Trachea	13 (54.2)	2 (66.7)	0 (0)	1 (100)	0.6
Catheter	4 (16.7)	0 (0)	0 (0)	0 (0)	
Urine	5 (20.8)	1 (33.3)	0 (0)	0 (0)	
Wound	2 (8.3)	0 (0)	1 (100)	1 (100)	

IMP: Imipenemase, VIM: Verona integron-encoded metallo-b-lactamase, OXA: Oxacillinase

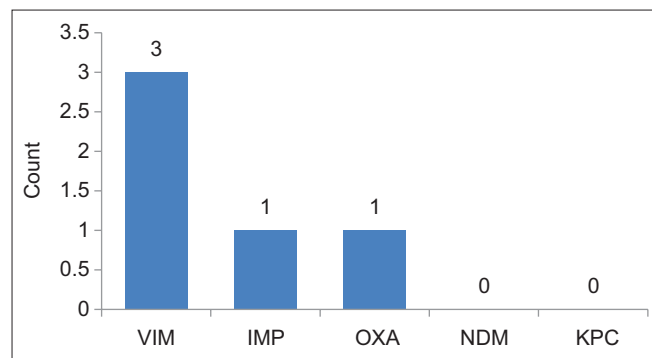


Figure 1: Frequency distribution of genes expression in 29 cases of *Klebsiella pneumoniae*

from patients admitted in ICUs in Al-Zahra Hospital during 2011–2012.

The blaKPC is the most common carbapenemase in the United States.^[7] Several reports described hospital outbreaks in the North-eastern United States involving *K. pneumoniae* carrying KPC.^[8,9] KPC-harboring isolates have also been increasingly recovered from other parts of the world, including Europe,^[10] Asia,^[11,12] and South America.^[13] KPC producing *K. pneumoniae* isolates have been reported from Tehran, Iran.^[14]

The blaNDM has been reported from India, Pakistan, and other parts of Asia, Europe, North America, and Australia.^[15,16] In 2013, Shahcheraghi *et al.*, reported first case of NDM-producing *K. pneumoniae* in Tehran, Iran.^[17]

In our study, the blaKPC and blaNDM were not identified so far, because of rapid progression rates of these genes, it is necessary to monitor the epidemiologic changes of these carbapenemase genes in *K. pneumoniae* in our hospital. One study^[18] reported high frequency of blaKPC gene in *K. pneumoniae* in Al-Zahra Hospital, but they used nonmolecular methods and genes can only be detected by molecular methods.

The blaVIM and blaIMP genes have been described in Asia, Europe, North America, South America, and Australia.^[19,20] According to a study of Zeighami *et al.* in 2015 in Zanjan, Iran, metallo-b-lactamase producing *K. pneumoniae* strains carried bla_{IMP} and bla_{VIM} were found in 100% and 41.6%, respectively.^[21]

The blaOXA was identified in Turkey, Europe, the Middle East, and Northern Africa.^[22] In 2013, Azimi *et al.*, reported first case of OXA-producing *K. pneumoniae* in Iran (Azimi).

In our study, blaIMP, blaVIM, and blaOXA were identified in the minority (17.2%) of samples. Thus, other carbapenemase producing genes should be investigated.

It is suggested that further studies with more samples should be considered to investigate the existence of genes

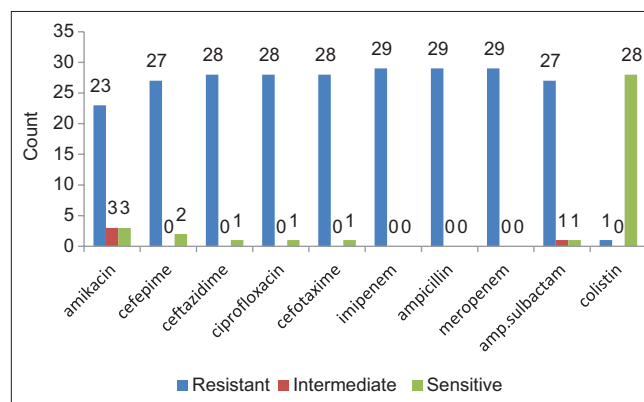


Figure 2: Frequency distribution of antibiotic resistance of *Klebsiella pneumoniae* samples

containing carbapenemases. By recognition of resistant organisms and prescribing suitable antibiotics, we can decrease the mortality and morbidity of hospitalized patients in ICUs and minimize the costs and duration of hospitalization.

In order to existence of blaIMP, blaVIM, and blaOXA genes in *K. pneumoniae* in our hospital and possibility of horizontal transmission to other bacteria, more attention should be paid to nosocomial infection control measures.

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Conflicts of interest

There are no conflicts of interest.

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