



Prevalence of Carbapenemase and blaKPC gene in *Klebsiella pneumoniae* Strains Isolated from Isfahan Hospitals, Iran

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ABSTRACT

Aims *Klebsiella pneumoniae* is a Gram-negative opportunistic pathogen. The carbapenems are effective therapeutic choice for the treatment of *Klebsiella pneumoniae* infections. Carbapenemases are a group of enzymes capable of hydrolyzing carbapenems. This study was to introduce phenotypic and genotypic methods to identify the carbapenemase-producing isolates of *Klebsiella pneumoniae*.

Materials & Methods this study was to introduce phenotypic and genotypic methods to identify the carbapenemase-producing isolates of *Klebsiella pneumoniae*. The Modified Hodge Test (MHT) was performed to determine the susceptibility of isolates to antibiotics. The final products of PCR were electrophoresed on agarose gel.

Findings The highest rate of resistance were observed for piperacillin (84%) and the lowest for ertapenem (50%). The majority of MHT positive isolates was from urine (64.7%), while abdominal and cerebrospinal fluids (0%) were the lowest. In addition, the ICU wards with 47 (69.1%) and the emergency units with 4 (5.9%) samples, had the most and the least frequent cases, respectively. MHT was positive in 68 *K. pneumoniae* isolates, but none of them were positive for blaKPC gene.

Conclusion The blaKPC gene has low prevalence in the Isfahan City, Iran.

Keywords *Klebsiella pneumoniae*; Carbapenemase; PCR; Prevalence

CITATION LINKS

[1] As nosocomial pathogens: Epidemiology, taxonomy, typing methods ... [2] Carbapenem-resistant *Klebsiella pneumoniae* harbouring ... [3] Novel carbapenem-hydrolyzing β -lactamase, KPC-1, from a ... [4] Predictors of carbapenem-resistant *Klebsiella pneumoniae* ... [5] Prevalence of qnr genes in extended-spectrum β -Lactamase producing *Klebsiella pneumoniae* isolated ... [6] Isolation of imipenem-resistant enterobacter species: Emergence of KPC-2 carbapenemase, molecular ... [7] Use of boronic acid disk tests to detect extended-spectrum β -lactamases in ... [8] In vivo acquisition of high-level resistance to ... [9] Current methods for the identification of ... [10] Carbapenemases: The versatile ... [11] Emerging carbapenemases: A global ... [12] Emergence of *Klebsiella pneumoniae* carrying blaVIM and ... [13] Emergence of carbapenem-resistant *Klebsiella* species possessing the class ... [14] Carbapenemases in *Klebsiella pneumoniae* and other ... [15] A study on prevalence of KPC producing from *Klebsiella* ... [16] Antimicrobial susceptibility testing: A review of general principles and ... [17] A simple test for penicillinase production ... [18] Identification of KPC-producing *Klebsiella* ... [19] High prevalence of KPC-2-type carbapenemase coupled with CTX-M-type extended-spectrum β -lactamases ... [20] The modified Hodge test is a useful tool for ... [21] Carbapenemase-producing *Klebsiella pneumoniae* ... [22] Detection of the *Klebsiella pneumoniae* ... [23] Frequency of *Klebsiella pneumoniae* producing ... [24] Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase ... [25] Ertapenem resistance among extended-spectrum- β -lactamase producing ... [26] Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary ... [27] The emergence of OXA-48- and ... [28] Multi-drug carbapenem-resistant *Klebsiella pneumoniae* infection carrying ... [29] Evaluation of phenotypic methods for detection of *Klebsiella* ... [30] Extended-spectrum β -lactamase and Carbapenemase production among burn and non-burn clinical ... [31] The frequency of blaVIM, blaIMP, blaKPC and blaNDM Carbapenemase genes in ... [32] Characterization of Carbapenem resistance mechanisms in *Klebsiella pneumoniae* and ... [33] blaKPC gene detection in clinical isolates of Carbapenem resistant ... [34] Emergence of serine Carbapenemases (KPC and SME) among clinical strains ... [35] Isolation and identification of Carbapenemase KPC producing strains ... [36] Carbapenemase-producing *Klebsiella pneumoniae* in ... [37] Antimicrobial activities of tigecycline and ...

Introduction

Klebsiella pneumoniae is a Gram-negative opportunistic pathogen. These bacteria can cause infections, e.g. septicemia, pneumonia, urinary tract infection (UTI), meningitis, diarrhea and soft tissue infection [1].

The increasing appearance of resistance to various antibiotics in *Klebsiella pneumoniae* isolates is worrisome. Carbapenems were first introduced in 1980 [2]. The carbapenems have an extremely broad spectrum of antimicrobial activity and are highly resistant to a variety of β -lactamases. Antimicrobial activity of carbapenem antibiotics is extremely high [3].

The carbapenems are effective therapeutic choice for the treatment of *Klebsiella pneumoniae* infections [4]. Many multi-drug-resistant hospital-acquired bacteria are often sensitive to carbapenems.

However, Emergence and spread of resistance to carbapenem antibiotics has been increased among these isolates and limited the suitable therapeutic choices for the treatment [5-7]. There are 3 mechanisms of resistance to carbapenems; 1-producing carbapenemase enzymes, 2-porin loss, and 3-expression of efflux pumps that the first one is the major threat [8].

Carbapenemases are a group of enzymes capable of hydrolyzing carbapenems, cephalosporins and broad-spectrum penicillin [9]. A number of carbapenemases have been reported as grouped; KPC, GES, SME, NMC-A, and IMI types (Ambler Class A), IMP, VIM, and NDM types (Ambler Class B), OXA type (Ambler Class D) [10, 11].

The KPCs are highly clinically important enzymes with the ability of resistance development between bacteria [12]. The KPCs are encoded by blaKPC gene. The first case of KPC-producing bacteria was reported in the United States and now is prevalent in Puerto Rico, Colombia, China, Argentina, and recently in the Middle East [3, 13, 14].

Considering the fact that information on the KPC is limited in Iran, the identification of these pathogens is a major challenge for diagnostic laboratories [15]. So this study was to introduce phenotypic and genotypic methods to identify the carbapenemase-producing isolates of *Klebsiella pneumoniae*.

Materials and Methods

This experimental cross-sectional study was carried out at Alzahra and Khorshid Hospitals of Isfahan City, Iran, in 2017. The clinical specimens such as tracheal aspirate, blood, urine, urethral catheter, wound, etc. were used to isolate samples of *K. pneumoniae*. All samples were cultured on a specific medium (TSI, SIM, MR VP, Simon Citrate,

Urea Agar) and finally 100 colonies with the characteristics of *K. pneumoniae* were isolated.

The Modified Hodge Test (MHT) was performed to determine the susceptibility of isolates to antibiotics; ceftazidime (30 μ g), imipenem (10 μ g), meropenem (10 μ g), trimethoprim sulfamethoxazole (30 μ g), gentamicin (10 μ g), aztreonam (30 μ g), ciprofloxacin (5 μ g), piperacillin (100 μ g), Ertapenem (10 μ g), cefotaxime (30 μ g), cefipime (30 μ g) using the Kirby-Bauer antimicrobial disk diffusion (Mast; England) according to the CLSI recommendation [16, 17]. At first the aliquot of *E. coli* ATCC® 25922™ (ATCC; United States) in 5ml saline was adjusted to 0.5McFarland standard, and the suspension was diluted 1:10. Next, the sterile cotton swab was dipped into the suspension and inoculated on Mueller-Hinton agar plate (Merck; Germany); then a 10 μ g meropenem disk was placed in the center of the plate. In a straight line, by a sterile swab, suspected bacteria (resistant or semi-susceptible isolates to one or more antibiotics of the carbapenem family and third generation cephalosporins), were streaked from the edge of the meropenem disc (MEM) to the plate edge. The plate was incubated overnight at 35 \pm 2°C in ambient air for 16-24 hours. In negative isolates the clear zones around the disk remains homogeneous, while carbapenemase-producing isolates cause cloverleaf like indentation.

The forward primer 5'-TCTGGACCGCTGGGAGCTGG-3' and reverse primer 5'-TGCCCCGTTGACGCCCAATCCC-3' were used to amplify 399bp of blaKPC gene in samples [18]. Polymerase chain reaction (PCR) was conducted in a final reaction volume of 30 μ l as follow: Initial activation at 95°C/10 minutes; 36 cycles of 94°C/60 seconds; 63°C/60 seconds; 72°C/60 seconds; and Final extension step of 72°C/5 minutes.

The final products of PCR were electrophoresed on agarose gel [18]. The extracted acid nucleics of *K. pneumoniae* ATCC®BAA-1705™ and *K. pneumoniae* ATCC®BAA-1706™ (ATCC; United States) were used as positive and negative controls, respectively.

Findings

62 *K. pneumoniae* samples were isolated from females and 38 from males (p=0.01). The urine isolates (46 samples) were the prevalent while the blood and cerebrospinal fluid derived samples (each with 2 samples) were the rare clinical cases. The ICU wards (53 samples) and the infant ward (7 samples) had the most and the least frequent cases, respectively (Table 1).

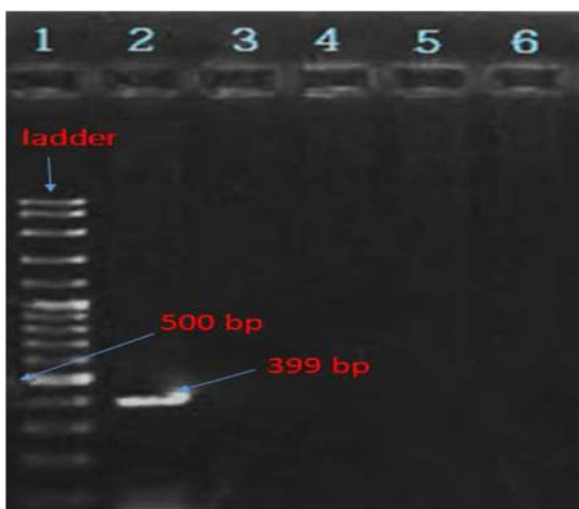
The highest rate of resistance were observed for piperacillin (84%) and the lowest for ertapenem (50%; Table 2).

Table 1) Number of *Klebsiella pneumoniae* isolates according to specimen type and clinical ward

Specimen Type	ICU	Internal	Surgery	Emergency	Infant
Urine (46)	36	5	0	0	5
Tracheal (16)	9	5	1	1	0
Catheter (8)	0	0	8	0	0
Wound (7)	0	2	3	2	0
Bronchial (7)	0	3	0	4	0
Abdominal fluid (5)	0	3	2	0	0
Abscess (4)	4	0	0	0	0
Sputum (3)	2	0	0	1	0
Cerebrospinal fluid (2)	1	0	0	0	1
Blood (2)	1	0	0	0	1
Total (100)	53	18	14	8	7

Table 2) Antimicrobial resistance profile of *Klebsiella pneumoniae* isolates

Antibiotics	Resistant	Intermediate	Sensitive
Cefepime	71	0	29
Ceftazidime	70	1	29
Cefotaxime	76	7	17
Gentamicine	62	1	37
Ciprofloxacin	70	6	24
Meropenem	66	6	28
Imipenem	64	12	24
Ertapenem	50	6	44
Piperacillin	84	7	9
Sulfamethoxazole-Trimethoprim	53	3	44
Aztreonam	71	7	22

**Figure 1)** Agarose gel Image of the blaKPC gene; Lane 1: Ladder, Lane 2: *K. pneumoniae* ATCC BAA-1705 (positive control), Lane 3-5: samples, Lane 6: *K. pneumoniae* ATCC BAA-1706 (negative control)

The majority of MHT positive isolates was from urine (64.7%), while abdominal and cerebrospinal fluids (0%) were the lowest.

In addition, the ICU wards with 47 (69.1%) and the emergency units with 4 (5.9%) samples, had the most and the least frequent cases, respectively. MHT was positive in 68 *K. pneumoniae* isolates, but none of them were positive for blaKPC gene (Figure 1).

Discussion

Carbapenems such as imipenem and meropenem are considered as the last line agents in treating severe infections caused by *Klebsiella pneumoniae*. However, the emergence and global outbreak of carbapenemase-producing *Klebsiella pneumoniae* led to resistance development against these antibiotics [2]. The method for phenotypic detection of carbapenemase-producing bacteria that presently approve by the CLSI is the Modified Hodge Test (MHT). Molecular approaches such as PCR are inevitable and necessary to determine the type of carbapenemase [19, 20].

In the current study, similar to Agha-Seyed Hosseini *et al.* [21], urinary specimens from the ICU wards were the most cases and similar to Bina *et al.* [22], the highest resistance was to piperacillin. In comparison to Bina *et al.* [22] and Roudbari *et al.* [23], our findings indicate higher prevalence of carbapenem-resistant *K. pneumoniae* isolates that can mean more frequency of carbapenemase in Isfahan Hospitals and needs further investigation and consideration.

In the present study, 68 isolates were identified as carbapenemase-producing strains of *Klebsiella pneumoniae* by MHT, but none of them were positive for blaKPC gene, which is similar to other studies [24-28]. Bina *et al.* have also reported all isolates negative for blaKPC gene [22]. In several other publications in Iran, all carbapenemase-producing strains were blaKPC negative [29-31].

According to Krishnappa *et al.* [32], 95% of *Klebsiella* isolates were carbapenem-resistant and Shanmugam *et al.* [33] have reported the frequency of carbapenemase-producing cases as 82%, which *K. pneumoniae* were the predominant isolate. In contrast, Deshpande *et al.* have reported 51 out of 8885 Enterobacteriaceae cases positive for carbapenemase [34]. The study of Agha-Seyed Hosseini *et al.* in Kashan City, Iran, have indicated 26.5% cases of *K. pneumoniae* isolates as imipenem-resistant. The isolates showed high resistance to ampicillin, cefalotin, and cefotaxime, while the low resistance was found to ertapenem and doripenem.

The urinary and respiratory samples from ICU departments accounted for the most frequent infections [21]. Roudbari *et al.* have reported 20 out of 280 isolates of *K. pneumoniae* from Qaem and Imam Reza Hospitals of Mashhad City, Iran, as positive for carbapenemase [23]. Bina *et al.* have reported the highest and the lowest resistance to piperacillin and imipenem, respectively [22]. Shokri *et al.* have reported that 87% of *K. pneumoniae* isolates were carbapenem-resistant [35]. According to Bratu *et al.* the blaKPC gene was found in 24% of carbapenem-resistant *K. pneumoniae* isolates [36]. The blaKPC positive isolates were reported 70.6 and 51% by Castanheira *et al.* [37] and Chen *et al.* [19].

There is a dramatic increase in carbapenem-resistant isolates in Iran, despite the absence of blaKPC gene. These findings indicate that other carbapenemase encoding genes must be considered for future studies. It is suggested that PCR must be considered for all carbapenemase encoding genes, if phenotypic methods are used.

Conclusion

The blaKPC gene has low prevalence in the Isfahan City, Iran, so other encoding genes for carbapenem hydrolyzing enzymes must be responsible for resistance to carbapenem family in *Klebsiella pneumoniae* isolates.

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important intellectual content (15%); Nasr Isfahani (Forth Author), Drafting of the manuscript, Critical revision of the manuscript for important intellectual content (15%).

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