International Journal of
Enteric Pathogens

Int J Enteric Pathog. 2018 May;6(2):36-40 Original Article

http://enterpathog.abzums.ac.ir doi 10.15171/ijep.2018.10

Evaluation of Antibiotic Resistance Pattern and Efficacy of Modified Hodge Test for Detection of Carbapenem-Resistant *Klebsiella pneumoniae* Strains Isolated From Clinical Samples

Leila Gheitani, Hossein Fazeli*, Sharareh Moghim, Bahram Nasr Isfahani

Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

***Corresponding Author:**
Hossein Fazeli, Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
Tel: +98-3137929038;
Email: h_fazeli@med.mui.ac.ir

Published Online March 8, 2018

Keywords: *Klebsiella pneumoniae*, Carbapenemase, Modified Hodge test

Abstract

Background: *Klebsiella pneumoniae* is a gram-negative opportunistic pathogen that causes different infections, however, in recent years the emergence of resistance to carbapenem antibiotic among these isolates has caused failure in the treatment of these infections.

Objective: The aim of this study was to evaluate the prevalence of carbapenemase-producing *K. pneumoniae* strains among various clinical specimens obtained from different wards of Isfahan hospitals, Iran.

Materials and Methods: In this cross-sectional study, 100 different clinical samples were collected from different wards of teaching hospitals in Isfahan, Iran. *K. pneumonia* isolates were identified by different standard biochemical tests. Antimicrobial susceptibility tests were performed as standard disk-diffusion based on the instructions of the Clinical Laboratory Standards Institute (CLSI). For detection of *K. pneumoniae* carbapenemase (KPC)-producing strains, isolates were investigated by the modified Hodge test (MHT) based on CLSI instructions.

Results: The study population included 62 females and 38 males ($P=0.01$). The highest and the lowest rates of resistance were observed for piperacillin (84%) and ertapenem (50%), respectively. The MHT was positive for 68 (68%) isolates from which the highest rates of resistance were observed for piperacillin (91.2%) and cefotaxime (83.8%).

Conclusion: This study demonstrated high prevalence of carbapenemase-producing *K. pneumoniae* isolates, which shows an urgent need to review and modify the pattern of antibiotic consumption. In addition, in future studies genotypic methods for all carbapenemase genes should be employed to determine the cause of the resistance.

Received December 16, 2017; **Revised** February 19, 2018; **Accepted** February 24, 2018

Background

Klebsiella pneumoniae is a gram-negative opportunistic pathogen that can cause infections such as pneumonia, septicemia, urinary tract infection (UTI), and soft tissue infection.[1,2] Carbapenems are a class of effective therapeutic agents for the treatment of these infections, however, in recent years resistance to carbapenems has been increased.[3,4] The mechanisms of resistance to carbapenems may be related to a series of causes including weakness in bacterial outer membrane permeability, increasing production of extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases and expression of betalactamases like carbapenemases.[5,6] The production of carbapenemases especially *K. pneumoniae* carbapenemase (KPC) is the most important mechanism of enzymatic resistance. Carbapenemase-producing *k. pneumoniae* are a group of emerging, highly drug-resistant bacilli, which cause infections associated with significant morbidity and mortality.[7] A number of carbapenemases have been reported including KPC, GES, SME, NMC-A and IMI types (Amber class A), IMP, VIM and NDM type (Amber class B), metallo-β-lactamases and OXA type (Amber class D), and oxacillinases.[8,9] KPC-producing *K. pneumoniae* was reported to emerge in some countries such as the northeastern USA, Greece, Israel, Columbia and Puerto Rico. Moreover, France, Sweden, Norway, Scotland, China, Colombia, Brazil, Trinidad and Tobago, and Poland are the countries that have reported pathogen-harbouring KPCs.[10,11] Information on this issue is limited in our country.

Objectives

The aim of this study was to evaluate the prevalence of carbapenemase-producing *K. pneumoniae* strains among various clinical specimens obtained from different wards of Isfahan hospitals, Iran.

Materials and Methods

Sampling

One hundred clinical samples including urine, tracheal, catheter, wound, bronchial, abdominal fluid, abscess, sputum, cerebrospinal fluid and blood were collected from different locations (ICU, internal medicine ward, surgery, emergency, infant ward) of chosen teaching hospitals (Alzahra and Khorshid) in Isfahan, Iran. *K. pneumoniae* isolates were detected by IMVIC standard biochemical tests (all samples were citrate-positive, nonmotile, Voges-Proskauer [VP]-positive, methyl red [MR]-negative, and lactose fermenters) and urease and then confirmed by detection of *ureD* gene, responsible for urea hydrolysis, by polymerase chain reaction (PCR) method. The forward primer 5'-CCCGTTTTACCCGGAAGAAG-3' and reverse primer 5'-GGAAAGAAGATGGCATCCTGC-3' were used to amplify 243 bp of *ureD* gene. PCR was conducted in a final reaction volume of 25 µL as follows: initial denaturation at 95°C/3 minutes, 30 cycles of 95°C/30 seconds (denaturation), 45°C/45 seconds (annealing), 72°C/60 seconds (elongation), and a final extension at 72°C/60 seconds. The final products of PCR were electrophoresed on agarose gel.

Antibiotic Susceptibility Test

The antimicrobial susceptibility was tested by the standard method, Kirby-Bauer disk-diffusion on Müeller-Hinton agar (Merck, Germany). The performance and interpretation were given based on the instructions of the Clinical Laboratory Standards Institute (CLSI). All antibiotic disks including ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), trimethoprim sulfamethoxazole (30 µg), gentamicin (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), piperacillin (100 µg), ertapenem (10 µg), cefotaxime (30 µg), and cefipime (30 µg) were purchased from Mast, England. Standard isolate of *E. coli ATCC25922* was used as a quality control strain.

Modified Hodge Test

The modified Hodge Test (MHT) was performed according to the CLSI recommendations. At first, the aliquot of *E. coli* ATCC 25922 in 5 mL saline was adjusted to 0.5 McFarland standard, and then the suspension was diluted 1:10. Next, the sterile cotton swab was dipped into the suspension and inoculated on Müeller-Hinton agar plate, then a 10 µg 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±2°C in ambient air for 16–24 hours. In negative isolates, the clear zones around the disk remain homogeneous, while carbapenemase-producing isolates cause cloverleaf like indentation.

Results

The samples tested comprised 100 clinical samples of *K. pneumoniae* that were identified by biochemical tests and also were confirmed by determination of the *ureD* gene. Analysis for presence of *ureD* gene demonstrated that all isolates were positive for *ureD* gene which confirmed their identity as *K. pneumoniae* (Figure 1).

Among 100 clinical isolates of *K. pneumonia,* 62 were females and 38 were males ($P=0.01$). The highest prevalence was related to the urine specimens with 46 (46%) isolates, while blood and cerebrospinal fluid-derived samples each with 2 (2%) were the rare ones. The clinical profile of *K. pneumoniae* isolates are demonstrated in Table 1. The ICU ward with 53 (53%) and the infant ward with 7 (7%) samples were the most and the least frequent cases, respectively.

Results of antibiotic susceptibility test are shown in Table 2. The highest and the lowest rates of resistance were observed for piperacillin (84%) and ertapenem (50%), respectively.

The MHT was performed for suspected carbapenemase-producing isolates (Figure 2).

The MHT was positive for 68 (68%) isolates. Urine samples (64.7%) accounted for the majority of cases, while abdominal and cerebrospinal fluids (0%) were the lowest frequent groups.

Based on the results (Table 3), the ICU wards with 47 (69.1%) and the emergency wards with 4 (5.9%) samples were the most and the least frequent cases in MHT positive group, respectively.

Based on the results (Table 4), the highest rates of resistance were observed for piperacillin (91/2%) and cefotaxime (83/8%) in MHT positive group.

Discussion

Klebsiella pneumoniae is a gram-negative opportunistic pathogen of nosocomial infections that can remain on environmental surfaces and on human skin and respiratory tract.[12,13] The increasing appearance of resistance to various antibiotics in *K. pneumoniae* isolates

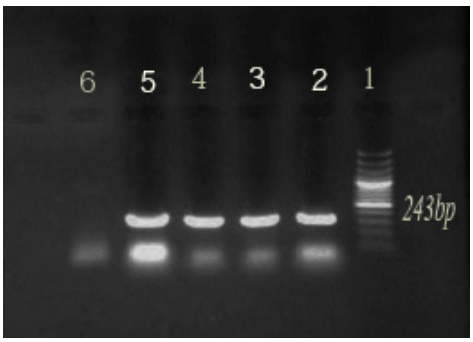

Figure 1. Agarose Gel Image of the *ureD* Gene (Lane 1: Ladder, Lane 5: Positive control, Lane 6: Negative control, Lane 2-4: Samples, Negative control: *E.coli* ATCC 25922, Positive control: *K. pneumoniae* ATCC700603)

Table 1. Prevalence of *Klebsiella Pneumoniae* Isolates by Type of Clinical Specimens

Specimen Type	No. of Patients (%)
Urine	46 (46)
Tracheal	16 (16)
Catheter	8 (8)
Wound	7 (7)
Bronchial	7 (7)
Abdominal fluid	5 (5)
Abscess	4 (4)
Sputum	3 (3)
Cerebrospinal fluid	2 (2)
Blood	2 (2)
Total	100 (100)

Table 2. Antimicrobial Resistance Profile of *Klebsiella Pneumoniae Isolates*

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

is worrisome. This study was designed to evaluate the prevalence of carbapenemase-producing *K. pneumoniae* strains among various clinical specimens obtained from different wards of Isfahan hospitals, Iran. In our study, 68% of isolates were positive as carbapenemase-producing. The urinary specimens from the ICU wards were the most frequent cases and the highest resistance was to piperacillin. In our findings, there was a raise in the resistant of *K. pneumoniae* isolates, especially carbapenem resistant strains in our city; this means that the frequency of carbapenemase is higher in Isfahan and needs further

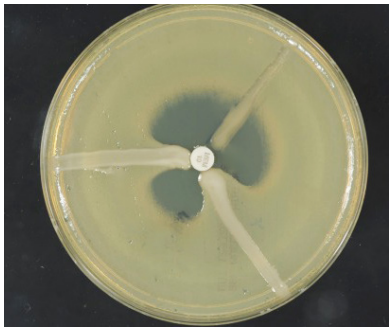

Figure 2. Isolates Tested for KPC Production Through MHT.

investigation and consideration. Although tests other than MHT, such as the aminophenylboronic acid and dipicolinic acid tests, may also be suitable options for the phenotypic screening of carbapenemases, the facilities necessary for these tests are not routinely available in the majority of laboratories. In this study, MHT was a suitable method for approving carbapenemase production. In Italy, 84% (32 of 38) of clinical isolates showed the production of carbapenemase.[14] In several other studies like that of Krishnappa et al and Shanmugam et al, the frequency of carbapenemase-producing cases were 82% (38/46) and 95%, respectively.[15,16] In another study in Brazil, 36 of the 44 carbapenem non-susceptible *K. pneumoniae* isolates were phenotypic carbapenemase producers as determined by the MHT.[17] In the studies conducted in Iran like that of Roudbari et al and Shokri et al, the prevalence of carbapenem-resistant *K. pneumoniae* were

Table 4. Antimicrobial Resistance Profile of *Klebsiella Pneumoniae* MHT Positive

Antibiotic Name	Susceptible (%)	Intermediate (%)	Resistant (%)
Cefepime	25	0	75
Ceftazidime	25	1.5	73.5
Cefotaxime	8.8	7.4	83.8
Gentamicine	33.8	1.5	64.7
Ciprofloxacin	20.6	7.3	72.1
Meropenem	19.1	7.4	73.5
Imipenem	23.5	10.3	66.2
Ertapenem	29.4	8.8	61.8
Piperacillin	2.9	5.9	91.2
Sulfamethoxazole-Trimethoprim	41.2	2.9	55.9
Aztreonam	17.6	11.8	70.6

Table 3. Prevalence of *Klebsiella pneumoniae* MHT Among the Clinical Locations

Clinical Ward		ICU	Internal	Surgery	Emergency	Infant	Total
MHT	+	47 (69.1%)	6 (8.8%)	5 (7.4%)	4 (5.9%)	6 (8.8%)	68
	-	6 (18.8%)	12 (37.5%)	9 (28.1%)	4 (12.5%)	1 (3.1%)	32

87% and 7.1%, respectively.[18,19] These differences show that hospitalization for a long time and the widespread use of broad-spectrum cephalosporins and carbapenems can increase carbapenemase-producing K. pneumoniae infections. The study of Agha-Seyed Hosseini et al in Kashan, Iran, indicated that among 181 K. pneumoniae isolates, 26.5% of cases were imipenem-resistant and 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 the ICU wards accounted for the most frequent infections.[20] Bina et al reported that the highest and the lowest resistance were to piperacillin and imipenem.[21] In our study, the results for the most frequent clinical samples and the highest resistance were similar to the results of studies of Agha-Seyed Hosseini et al and Bina et al, respectively. In comparison to the studies of Bina et al[21] and Roudbari et al,[18] in our study there was a raise in carbapenem-resistant strains, meaning that in our regions, there is a need for further guidance/information on infection prevention and for control team. Moreover, if phenotypic methods are used, we suggest the laboratories to employ PCR for all carbapenemase-encoding genes. We hope the results of this study be useful in the application of an effective control approach on infectious diseases to avoid and decrease the prevalence of carbapenem-resistant *K. pneumoniae*.

Authors' Contributions

Study concept and design: HF; Acquisition of data and sampling: LG; Analysis and interpretation of data: LG; Drafting the manuscript: LG; Critical revision of the manuscript for important intellectual content: LG; Study supervision: HF.

Ethical Approval

Not applicable.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Financial Support

This study was supported by Isfahan University of Medical Sciences (grant No: 395959).

Acknowledgments

Authors would like to thank the staff of Isfahan Antimicrobial Resistance Research Center and microbiology group of Isfahan university of medical science for supporting this study.

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