Immunopathol Persa. 2023;9(1):e34407 Original

Immunopathologia Persa

DOI:10.34172/ipp.2022.34407

http www.immunopathol.com

Prevalence of carbapenem-resistant gram-negative bacilli producing carbapenemase by modified carbapenem inactivation method in an educational hospital in Tehran

Mihan Pourabdollah[1], **Elham Askari**[1], **Leila Mansoury**[2*], **Hamze Mansoury**[3]

[1]Chronic Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Pathology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Generla Practitionaire, Lorestan University of Medical Sciences, Lorestan, Iran

***Correspondence to**
Leila Mansoury,
Email: l.mansoury@yahoo.com

Received 20 May 2022
Accepted 25 Aug. 2022
Published online 22 Oct. 2022

Keywords: Gram-negative bacilli, Bacterial resistance, Carbapenems, Carbapenemase enzyme, mCIM

Abstract

Introduction: Regarding the crucial role of geographic factors in different mechanisms underlying bacterial antibiotic resistance worldwide, it is necessary to design and conduct studies to determine the prevalence and specific underlying mechanisms of this phenomenon.

Objectives: This study was performed to assess the prevalence of carbapenem-resistant gram-negative bacilli which produce carbapenemase, in Loghman Hakim hospital, Tehran.

Patients and Methods: In this cross-sectional study, antibiotic resistance of 300 samples of gram-negative bacilli from different patients was evaluated; 145 of which were identified as carbapenem-resistant. Carbapenemase enzyme production in these samples was assessed by the modified carbapenem inactivation method (mCIM).

Results: About 81% of the samples were collected from the intensive care unit. In terms of sample type, most samples were obtained from trachea and urine culture. *Acinetobacter baumannii* (43%) was the most common carbapenem-resistant strain. *Klebsiella pneumoniae* (38%) and *Pseudomonas aeruginosa* (11%) ranked as the second and third most common strains, respectively. Based on mCIM evaluation, 82% of carbapenem resistance was due to the presence of carbapenemase enzyme which showed no significant difference neither between the both genders nor in various sample types. However, among carbapenemase-resistant bacilli, the presence of carbapenemase enzyme was significantly higher in *A. baumannii* (92%) and *Escherichia coli* (80%) and also in patients older than 50 years old.

Conclusion: The findings of the present study showed that half of the collected gram-negative bacilli were resistant to carbapenem, of which 82% was due to the carbapenemase enzyme. The presence of the carbapenemase enzyme was higher in older patients as well as in *Acinetobacter baumannii* and Escherichia coli strains.

Citation: Pourabdollah M, Askari E, Mansoury L, Mansoury H. Prevalence of carbapenem-resistant gram-negative bacilli producing carbapenemase by modified carbapenem inactivation method in an educational hospital in Tehran. Immunopathol Persa. 2023;9(1):e34407. DOI:10.34172/ipp.2022.34407.

Introduction

Antibiotic-resistant bacteria have been identified as one of the most important health threats, accounting for a high percentage of hospital mortality. Due to the high prevalence of gram-negative bacilli and their major role in nosocomial infections, their multiple drug resistance is of paramount importance both in developed and developing countries (1). Antibiotic resistance has recently hindered the treatment, increasing the mortality rate (2).

Among gram-negative bacilli, the high-prevalence Enterobacteriaceae family plays a key role (3). *Klebsiella* is one of the members of this family and one of the most common nosocomial pathogens with a great mortality rate. This bacterium can cause various health issues such as pneumonia, septicemia, diarrhea, purulent abscesses of various organs such as the liver, endophthalmitis, meningitis, urinary tract infection, and bacteremia. *Klebsiella* is part of normal flora and about one-third of people carry it in their intestines. In addition, the high rate of establishment of this bacterium in hospitalized patients, its high resistance to

Key point

In this cross-sectional study, antibiotic resistance of 300 samples of gram-negative bacilli was evaluated; 145 of which were identified as carbapenem-resistant. Modified carbapenem inactivation method (mCIM) showed that 82% of this resistance was due to the carbapenemase enzyme production, which was higher in older patients as well as in *Acinetobacter baumannii* and *Escherichia coli* strains.

Copyright © 2023 The Author(s); Published by Nickan Research Institute. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

various antibiotics, and rapid spread in various wards, especially the neonatal ward, are significant points of this organism. *Pseudomonas aeruginosa*, the third leading cause of nosocomial infections after Staphylococcus aureus and Escherichia coli, is most commonly reported in burns, urinary tract infections, and lung diseases such as cystic fibrosis (4).

Although gram-negative bacteria are inherently resistant to penicillin and most beta-lactam antibiotics, they are sensitive to piperacillin, ciprofloxacin, tobramycin, and imipenem (5). Carbapenems belong to the family of beta-lactams, showing the greatest effect on gram-negative and positive bacteria. As a broad-spectrum antibiotic, carbapenems are highly resistant to most betalactamases due to their molecular structure. Therefore, they can affect several pathogenic species with low side effects (6). However, with the widespread use of antibiotics, multidrug-resistant strains have become increasingly common (7). The emergence of resistance to carbapenems has been increasingly reported among Enterobacteriaceae, which can cause serious problems in the efficacy of these antibiotics in the treatment of severe infections (8). Various mechanisms are involved in the resistance to carbapenems among which gene expression regulation, formation of protective biofilm against host immune system and production of carbapenemase enzyme can be mentioned (8,9).

Specific carbapenemases can be globally found in many species of bacteria and are usually distributed in different regions or countries. However, due to the extent of traveling and the availability of medical care, the prevalence and mechanism of bacterial resistance may even vary in a particular country or region. Therefore, it is necessary to design and conduct studies in every single region to determine the prevalence and mechanisms of bacterial resistance for adopting necessary treatment methods on time.

Objectives

The present study aimed to investigate the prevalence of carbapenem-resistant gram-negative bacilli producing carbapenemase in an educational hospital.

Patients and Methods

Study design

This cross-sectional study was performed in 2020 in patients referring to Loghman Hakim hospital, Tehran. Three hundred gram-negative bacilli samples were isolated from blood, urine, sputum, and tracheal aspiration of patients admitted to various wards and identified according to standard laboratory methods. Each bacterium was first brought to the McFarland standard 0.5 and cultured on grass inside Muller-Hinton agar medium, while antibiogram discs of meropenem and imipenem were placed on the medium. The medium was kept in a 35±1°C incubator for 24 hours. The growth-inhibition zones around the discs were then recorded based on the Clinical and Laboratory Standards Institute (CLSI) criteria. Insensitivity (resistance or semi-susceptibility) to one or more carbapenem antibiotics was used for the early detection of bacteria with carbapenemase enzyme. Accordingly, 145 samples were resistant to carbapenem antibiotics.

The production of carbapenemase enzyme by susceptible bacteria was investigated using the modified Carbapenem Inactivation Method (mCIM) on the blood agar medium. With the help of a loop, Enterobacteriaceae at a rate of 1 µL and *Pseudomonas aeruginosa* and Acinetobacter baumannii at a rate of 10 µL were removed and their suspensions were prepared in 2 mL of trypticase soy broth (TSB) medium. A 10 µg meropenem antibiotic disc was then placed in suspension and incubated for 4 hours at 35°C. Before incubation, a suspension was prepared from standard *E. coli* ATCC 25922 bacterium at a concentration of 0.5 McFarland which was swabbed on the surface of Müller-Hinton agar after 15 minutes. The meropenem antibiotic disc was then removed from the TSB medium and placed in the center of the Müller-Hinton agar medium.

Inhibition zone diameter of meropenem-susceptible *E. coli* cultured on Müller-Hinton medium equal to 6-15 mm or small single colonies growing in 16-18 mm growth halo around meropenem disk implied carbapenemase production. The hydrolysis of meropenem by the isolate and the mCIM test was considered positive. The growth inhibition zone diameter greater or equal to 19 mm implied no production of carbapenemase and no hydrolysis of meropenem by the isolate, as a result, inhibition of meropenem-sensitive *E. coli* growth. Therefore, the mCIM test was considered negative. The patient’s age, gender, ward, sample type, cultured bacteria and mCIM status were recorded in a form.

Statistical analysis

Data were analyzed using SPSS version 25 software. Frequency and percentage were used to describe the variables. The chi-square test was used to compare the bacterial prevalence and mCIM status in different characteristics where $P < 0.05$ was considered the level of significance.

Results

Table 1 shows patient characteristics, samples, and mCIM status. Two-thirds of the patients were male, mostly over 50 years old. 81% of the samples were from the intensive care unit. In terms of sample type, most samples were from trachea and urine. The most common carbapenem-resistant bacilli were Acinetobacter baumannii (43%), *Klebsiella pneumoniae* (38%), and *Pseudomonas aeruginosa* (11%), respectively. Based on mCIM status, 82% of carbapenem-resistance cases were due to the presence of the carbapenemase enzyme.

Table 1. Patient characteristics, samples and mCIM test result

Variable		No (%)
Gender	Male	97 (67)
	Female	48 (33)
Age group (y)	<30	18 (12)
	30 to 50	36 (25)
	50 to 70	47 (33)
	>70	44 (30)
Hospital ward	ICU	118 (81)
	Lung	4 (3)
	Neurology	5 (3)
	Children	1 (1)
	Emergency	2 (1)
	Infectious	6 (4)
	Internal	4 (3)
	Heart	5 (4)
Sample type	Trachea	82 (57)
	Sputum	4 (3)
	Urinary catheter	8 (5)
	Blood culture	17 (12)
	Urine culture	33 (22)
	Pleural fluid	1 (1)
Bacteria	*Acinetobacter baumannii*	62 (43)
	Klebsiella pneumoniae	55 (38)
	Pseudomonas aeruginosa	16 (11)
	Escherichia coli	10 (7)
	Proteus vulgaris	2 (1)
mCIM result	Positive (with carbapenemase enzyme)	119 (82)
	Negative	26 (18)

mCIM: modified Carbapenem Inactivation Method.

Table 2 shows the prevalence of carbapenem-resistant bacteria in terms of gender, age groups, and sample type. In general, the prevalence of *K. pneumoniae* was approximately equal between the sexes, *Pseudomonas* and *Proteus* were observed only in men, while *Acinetobacter* and *E. coli* were more common in men ($P=0.020$). In terms of age groups, *Pseudomonas* was more common in the patients younger than 50, while other bacteria were more common in the age group older than 50 years ($P=0.046$). Regarding sample type, *Acinetobacter, Klebsiella*, and *Pseudomonas* were more present in pulmonary samples whereas *E. coli* and *Proteus* were more found in urine samples ($P=0.007$).

Table 3 shows the presence of the carbapenemase enzyme based on the positive mCIM regarding of gender, age groups, sample type, and carbapenem resistance. The presence of carbapenemase enzyme showed no significant difference neither between the sexes nor in various sample types, while it was significantly higher in people older than 50 years. In addition, among carbapenem-resistant bacteria, *Acinetobacter baumannii* (92%) and *E. coli* (80%) were significantly more abundant than others ($P=0.036$).

Discussion

The findings of the present study showed that out of a total of 300 gram-negative bacilli cultured, 145 (48%) were resistant to carbapenem. The most common resistant bacilli were *A. baumannii* (43%), *K. pneumoniae* (38%), and *P. aeruginosa* (11%). Based on mCIM result, the presence of carbapenemase enzyme was the most common resistance mechanism (82% of cases). Carbapenem resistance due to carbapenemase was higher in *A. baumannii* (92%) and *E. coli* (80%) and also in patients older than 50 years old.

Carbapenems are beta-lactam antibiotics resistant to hydrolysis by most beta-lactamases with a wide range of activities. They are commonly used to treat infections caused by multidrug-resistant pathogens (10-12). The administration of carbapenems has been incremented since 2000, following the spread of beta-lactam-resistant *E. coli* species other than carbapenems. This enhanced the number of beta-lactamase-producing species that also hydrolyzed carbapenems (carbapenemase) (13-15). Resistance to carbapenems can be through three mechanisms: reduced uptake of carbapenem, expelling it from the cell, or acquiring the carbapenemase gene, which is the main mechanism is carbapenemase production (16).

Resistance to carbapenems among gram-negative bacteria is mainly associated with carbapenemase production. Carbapenemase-producing gram-negative bacilli can cause a wide range of infections, including bacteremia, endocarditis, wound infections, urinary tract

Table 2. Comparison of the prevalence of carbapenem-resistant bacteria in gender, age group and sample type

	Acinetobacter baumannii	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus vulgaris*	*P* value*
Gender						
Male (97 people)	41 (66%)	31 (56%)	16 (100%)	7 (70%)	2 (100%)	0.020
Female (48 people)	21 (34%)	24 (44%)	0 (0%)	3 (30%)	0 (0%)	
Age group (y)						
< 50 (54 people)	24 (39%)	21 (38%)	9 (56%)	0 (0%)	0 (0%)	0.046
>50 (91 people)	38 (61%)	34 (62%)	7 (44%)	10 (100%)	2 (100%)	
Sample type						
Pulmonary samples (trachea, sputum, pleural fluid) (81 samples)	47 (76%)	26 (48%)	11 (69%)	3 (30%)	0 (0%)	0.007
Urine samples (catheter, culture) (41 samples)	10 (16%)	20 (36%)	3 (19%)	6 (60%)	2 (100%)	
Blood culture (17 samples)	5 (8%)	9 (16%)	2 (12%)	1 (10%)	0 (0%)	

* Chi-square test.

Table 3. Status of carbapenemase enzyme in carbapenem-resistant bacteria

	mCIM +	*P* value*
Gender		
Male (97 people)	82 (85%)	0.271
Female (48 people)	37 (77%)	
Age group (y)		
<50 (54 people)	39 (72%)	0.017
>50 (91 people)	80 (88%)	
Sample type		
Pulmonary samples (trachea, sputum, pleural fluid) (81 samples)	74 (85%)	0.205
Urine samples (catheter, culture) (41 samples)	30 (73%)	
Blood culture (17 samples)	15 (88%)	
Bacteria		
Acinetobacter baumannii (62 cases)	57 (92%)	0.036
Klebsiella pneumoniae (55 cases)	43 (78%)	
Pseudomonas aeruginosa (16 cases)	10 (63%)	
Escherichia coli (10 cases)	8 (80%)	
Proteus vulgaris (2 cases)	1 (50%)	

mCIM: modified Carbapenem Inactivation Method.
* Chi-square test.

infections and nosocomial pneumonia, often associated with high mortality, treatment failure, and prolonged hospitalization. Various studies have shown that most of the bacterial strains are developing resistance to most antibiotics, since these multidrug-resistant strains are rapidly spreading among hospitalized patients too (9,17).

Pseudomonas aeruginosa, *A. baumannii*, and *K. pneumoniae* are the most common antibiotic-resistant bacteria in intensive care unit (ICU) and other wards (18). Our study also showed that these three types of bacteria were the most common bacteria isolated from different samples which were resistant to carbapenems. In cases of nosocomial infections caused by these bacteria, the level of resistance to various antibiotics, especially carbapenems, has increased in recent years. However, the degree of resistance varies in different geographical areas, sections, and sample types. It has been also suggested that a large percentage of carbapenem resistance is due to the presence of the carbapenemase enzyme. In Greece, Feretzakis et al assessed antibiotic resistance of strains isolated from ICU and other wards and reported that *P. aeruginosa* and *K. pneumoniae* strains in ICU were resistant to carbapenems in 55% and 48% of cases, respectively. They were reported that almost all strains of *A. baumannii* (98%) in the ICU and other wards were resistant to most antibiotics, including carbapenems (18). Regarding the antibiotic susceptibility of bacteria isolated from ICU patients in Taiwan, Lai et al reported 23% and 63% cases of carbapenems-resistance in *P. aeruginosa* and *A. baumannii*, respectively (19).

In our study, the presence of carbapenemase in carbapenem-resistant species was higher in patients older than 50 years, which could be due to their weakened immune system, underlying diseases, and frequent hospitalizations.

In line with other studies, our study showed that approximately half of the bacteria isolated from different samples of hospitalized patients were carbapenem-resistant. The most mechanism was the presence of carbapenemase. *A. baumannii, K. pneumonia*, and *P. aeruginosa* were the most important bacteria with this enzyme, respectively. As an important class of antibiotics, carbapenems are administered for infections caused by multidrug-resistant microorganisms which has dramatically increased the resistance to them worldwide. Therefore, it is necessary to use these antibiotics as a last resort, only in the intensive care units of hospitals and prescribed only under close medical supervision to prevent their overuse. It is also necessary to monitor the antibiotic susceptibility of bacteria in each area continuously to take preventive measures against the development of antibiotic resistance.

Conclusion

The findings of the present study showed that half of the gram-negative bacilli isolated from different patient samples in various hospital wards were resistant to carbapenem. The presence of the carbapenemase enzyme was the main underlying mechanism of the resistance (82% cases). Carbapenem-resistance was more found in older patients and also in *A. baumannii* and *E. coli* strains. Since carbapenems are mainly used for infections caused by multidrug-resistant microorganisms, the emergence of resistance to them can be a warning sign of their overuse and inappropriate prescription. Therefore, their use should be limited to specific cases and under close supervision.

Limitations of the study

Low sample size and conducting the study only in one center are limitations of our study, which may prevent generalization of results. Therefore, it is recommended that such studies be conducted with higher sample sizes, more diverse samples, and also in multiple centers therefore, the results can be used in the development of local guidelines.

Authors' contribution

Conceptualization: MP, LM.
Methodology: MP, EA, LM.
Validation: MP, EA, LM, HM.
Formal analysis: LM.
Investigation: LM.
Resources: MP, LM.
Data curation: MP, LM.
Writing—original draft preparation: LM.
Writing—review and editing: MP, EA, LM, HM.
Supervision: MP.
Project administration: MP, LM.
Funding acquisition: LM.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study (IR.SBMU.MSP.REC.1398.287). This study was extracted from residency thesis of Leila Mansoury at this university (Thesis #16747). Besides, ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

Funding/Support

The authors declared no receipt of the financial support for the research, authorship, and/or publication of this paper.

References

1. Arvanitidou M, Katikaridou E, Douboyas J, Tsakris A. Prognostic factors for nosocomial bacteraemia outcome: a prospective study in a Greek teaching hospital. J Hosp Infect. 2005;61:219-24. doi: 10.1016/j.jhin.2005.03.006.
2. Kollef MH, Torres A, Shorr AF, Martin-Loeches I, Micek ST. Nosocomial Infection. Crit Care Med. 2021;49:169-87. doi: 10.1097/ccm.0000000000004783.
3. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 2016;3:15-21. doi: 10.1177/2049936115621709.
4. Carroll KC, Butel JS, Morse SA. Jawetz Melnick & Adelbergs Medical Microbiology. 27th ed. McGraw-Hill Education; 2015.
5. Rajat RM, Ninama G, Mistry K, Parmar R, Patel K, Vegad MJNJMR. Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a tertiary care hospital, Ahmadabad. Natl J Med Res. 2012;2:156-9.
6. Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. Antimicrob Agents Chemother. 2011;55:2420-3. doi: 10.1128/AAC.01452-10.
7. Chanawong A, M'Zali FH, Heritage J, Lulitanond A, Hawkey PM. SHV-12, SHV-5, SHV-2a and VEB-1 extended-spectrum beta-lactamases in Gram-negative bacteria isolated in a university hospital in Thailand. J Antimicrob Chemother. 2001;48:839-52. doi: 10.1093/jac/48.6.839.
8. Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother. 2012;56:559-62. doi: 10.1128/AAC.05289-11.
9. Ktari S, Mnif B, Louati F, Rekik S, Mezghani S, Mahjoubi F, et al. Spread of Klebsiella pneumoniae isolates producing OXA-48 β-lactamase in a Tunisian university hospital. J Antimicrob Chemother. 2011;66:1644-6. doi: 10.1093/jac/dkr181.
10. Lee Y, Bradley N. Overview and Insights into Carbapenem Allergy. Pharmacy. 2019;7:110. doi: 10.3390/pharmacy7030110.
11. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: here is the storm! Trends Mol Med. 2012;18:263-72. doi: 10.1016/j.molmed.2012.03.003.
12. Elshamy A, Aboshanab K, Yassien M, Hassouna N. Prevalence of carbapenem resistance among multidrug-resistant Gram-negative uropathogens. Archives of Pharmaceutical Sciences Ain Shams University. 2018;2:70-7. doi: 10.21608/aps.2018.18736.
13. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17:1791-8. doi: 10.3201/eid1710.110655.
14. Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol. 2015;13:42-51. doi: 10.1038/nrmicro3380.
15. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in *Klebsiella pneumoniae* and Other Enterobacteriaceae: an Evolving Crisis of Global Dimensions. Clin Microbiol Rev. 2012;25:682-707. doi: 10.1128/CMR.05035-11.
16. Elshamy AA, Aboshanab KM. A review on bacterial resistance to carbapenems: epidemiology, detection and treatment options. Future Sci OA. 2020;6:Fso438. doi: 10.2144/fsoa-2019-0098.
17. Neonakis IK, Spandidos DA, Petinaki E. Confronting multidrug-resistant *Acinetobacter baumannii*: a review. Int J Antimicrob Agents. 2011;37:102-9. doi: 10.1016/j.ijantimicag.2010.10.014.
18. Feretzakis G, Loupelis E, Sakagianni A, Skarmoutsou N, Michelidou S, Velentza A, et al. A 2-Year Single-Centre Audit on Antibiotic Resistance of Pseudomonas aeruginosa, *Acinetobacter baumannii* and *Klebsiella pneumoniae* Strains from an Intensive Care Unit and Other Wards in a General Public Hospital in Greece. Antibiotics. 2019;8. doi: 10.3390/antibiotics8020062.
19. Lai CC, Chen YS, Lee NY, Tang HJ, Lee SS, Lin CF, et al. Susceptibility rates of clinically important bacteria collected from intensive care units against colistin, carbapenems, and other comparative agents: results from Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART). Infect Drug Resist. 2019;12:627-40. doi: 10.2147/idr.S194482.