

## EVALUATION OF ZATARIA MULTIFLORA BOISS AND CARUM COPTICUM ANTIBACTERIAL ACTIVITY ON IMP-TYPE METALLO-BETA-LACTAMASE-PRODUCING PSEUDOMONAS AERUGINOSA

Fallah F.,<sup>1</sup> Taherpour A.,<sup>2</sup> Borhan R.S.,<sup>3</sup> Hashemi A.,<sup>3\*</sup> Habibi M.,<sup>3</sup> Sajadi Nia R.<sup>4</sup>

<sup>1</sup> Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>2</sup> Microbiology Department, Kurdistan University of Medical Sciences, Sanandaj, Iran

<sup>3</sup> Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>4</sup> Shahid Beheshti University of Medical Sciences, Tehran, Iran

**SUMMARY.** Carbapenem resistance due to acquired metallo-beta-lactamases (MBLs) is considered to be more serious than other resistance mechanisms. The aim of this study was to evaluate the antibacterial activity of *Zataria multiflora* Boiss and *Carum copticum* plants on IMP-producing *P. aeruginosa* strains. This experimental study was carried out on hospitalized burn patients during 2011 and 2012. Antibiotics and extracts susceptibility tests were performed by disc diffusion and broth microdilution methods. MBL detection was performed by Combination Disk Diffusion Test (CDDT). The bla(VIM) and bla(IMP) genes were detected by PCR and sequencing methods. Using Combination Disk Diffusion test method, it was found that among 83 imipenem resistant *P. aeruginosa* strains, 48 (57.9%) were MBL producers. PCR and sequencing methods proved that these isolates were positive for blaIMP-1 genes, whereas none were positive for bla(VIM) genes. The mortality rate of hospitalized patients with MBL-producing *Pseudomonas* infection was 4/48 (8.3%). It was shown that *Zataria multiflora* and *Carum copticum* extracts had a high antibacterial effect on regular and IMP-producing *P. aeruginosa* strains in 6.25 mg/ml concentration. The incidence of MBL-producing *P. aeruginosa* in burn patients is very high. In our study, all MBL-producing isolates carry the blaIMP-1 gene. Therefore, detection of MBL-producing isolates is of great importance in identifying drug resistance patterns in *P. aeruginosa*, and in prevention and control of infections. In this study, it was shown that extracts of *Z. multiflora* and *C. copticum* have high antibacterial effects on beta-lactamase producing *P. aeruginosa* strains.

**Keywords:** *P. aeruginosa*, Metallo-β-lactamases, *Zataria multiflora* Boiss, *Carum copticum*

### Introduction

Bacteria have the genetic ability to transfer and acquire drug resistance factors. In recent years, inappropriate use of antibiotics has led to drug resistance among bacteria, which is the cause of untreatable 'superbugs' and high death rates in clinics throughout the world, particularly among patients with suppressed immunity. It is reported that about 25,000 people in the E.U. and 63,000 in the U.S. die each year due to infectious diseases caused by multi-drug resistant (MDR) bacteria.<sup>1</sup> *P. aeruginosa* is the common cause of nosocomial infections<sup>2</sup> and, as an opportunistic pathogen, causes other infections such as pneumonia, septicemia, urinary tract infection, endocarditis, skin, ear and eye infections, as well as being a leading cause of morbidity and mortality among hospitalized burn patients.<sup>3</sup> Carbapenems are the last generation of drugs

for treatment of infections caused by MDR *P. aeruginosa*.<sup>4</sup> Carbapenems, including imipenem, meropenem and doripenem are the most potent antibacterial agents for the treatment of *P. aeruginosa* infections.<sup>5</sup> *P. aeruginosa* resistance to carbapenems is due to loss of OprD porin expression, high-level expression of AmpC enzymes, and increased expression of several efflux systems such as MexA–MexB–OprM. Among clinical isolates of *Pseudomonas spp.*, resistance to carbapenems has been found to be mainly due to the production of carbapenem-hydrolyzing enzymes such as metallo-β-lactamases (MBLs).<sup>6</sup> Carbapenem resistance due to acquired MBLs is considered to be more serious than other resistance mechanisms because MBLs can hydrolyze almost all beta-lactam antibiotics except monobactams. Furthermore, the MBL-encoding genes located on integrons can be disseminated easily from one bacterium to another.<sup>7</sup> Many MBLs

\* Corresponding author: Dr. Ali Hashemi, Ph.D, Department of Microbiology, Shahid Beheshti University of Medical Sciences, Koodakyar St., Tabnak Blv., Yaman Av., Chamran Highway, Tehran, Iran. P. Box: 19857-17443. Tel.: +9821 2387 2556 / +9891 2294 7439; e-mail: hashemi1388@yahoo.com / ali.hashemi@sbm.ac.ir

have been found in *P. aeruginosa*, including Imipenemase (IMP), São Paulo metallo (SPM), (Verona integron-encoded metallo- $\beta$ -lactamases (VIM)), Seoul imipenemase (SIM), Japan, Kyorin University Hospital Imipenemase (KHM), German imipenemase (GIM), New-Delhi metallo-beta-lactamase-1 (NDM-1) and Australian Imipenemase (AIM). The genes of both IMP and VIM-type in clinical isolates of *P. aeruginosa* are often encoded on mobile elements inserted into class 1 integrons.<sup>8</sup> The integrons are located on transposons or plasmids, the distribution of which contributes to the wide spread of this resistance mechanism.<sup>9</sup>

*Zataria multiflora* Boiss, a member of the Labiatae family, grows in countries such as Pakistan, Afghanistan and Iran. Traditionally, it has been used as a diuretic, an antiseptic, a flavoring, a carminative, and as an antispasmodic agent, as well as for premenstrual pain, edema, sore throat, jaundice, chronic catharsis and asthma treatment. *Z. multiflora* has been reported to have applicable medical properties including pain-relieving, immunostimulant, antibacterial, anticandidal, antifungal, antioxidant, antinociceptive, and anti-inflammatory effects.<sup>10-13</sup> *Carum copticum* grows in Egypt, East India and Iran, with bright flowers and brownish seeds which have a thymol-like odour. Its essential oil contains  $\alpha$ -pinene, paracymene, terpinene,  $\beta$ -pinene and other components such as thymol and carvacrol. The seeds can be used as a diuretic, analgesic, anti-asthma, anti-vomiting and anti-dyspnea agent. They also have a wonderful effect on skin and are good for neural and urinary tract disorders.<sup>14-16</sup>

Our study was conducted on hospitalized burn patients in Shahid Motahari Hospital, Tehran, Iran, from 2011 to 2012. The aims of this study were threefold: firstly, to determine the antibiotic resistance patterns of *P. aeruginosa*, secondly to detect bla<sub>VIM</sub> and bla<sub>IMP</sub> metallo-beta-lactamase genes, and thirdly to evaluate the effects of *Z. multiflora* and *Carum copticum* methanolic, acetonetic and chloroformic extracts on *P. aeruginosa* strains producing metallo- $\beta$ -lactamase (bla<sub>IMP</sub>) isolated from the studied patients.

## Materials and methods

### Isolation and Clinical Identification

From September to January 2011, 100 isolates of *P. aeruginosa* were collected by sterile swabs from 448 burn patients referred to Shahid Motahari Hospital (level I burn care center) in Tehran, Iran. Prior to sampling, the wounds were washed by Physiological Serum. Samples were transferred in Stuart media, cultured on Cetrimide and MacConkey agar and incubated at 37°C for 24 hours. Pigmented and odoriferous colonies were analyzed using biochemical tests including an oxidase test, a catalase sugar fermentation test and growth ability at 42°C. The isolates were stored at -20°C in brain heart broth containing

20% glycerol. *P. aeruginosa* ATCC27853 was used as a control strain.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility to imipenem (IPM, 10  $\mu$ g), meropenem (MEM, 10  $\mu$ g), ceftazidime (CAZ, 30  $\mu$ g), cefotaxime (CTX, 30  $\mu$ g), amikacin (AK, 30  $\mu$ g), tobramycin (TOB, 10  $\mu$ g), piperacillin/Tazobactam (PTZ, 100/10  $\mu$ g), ciprofloxacin (CIP, 5  $\mu$ g), cefepime (FEP, 30  $\mu$ g), ceftriaxone (CRO, 30  $\mu$ g), aztreonam (ATM, 30  $\mu$ g), gentamicin (GEN, 10  $\mu$ g) and carbenicillin (Car, 100  $\mu$ g) (Mast Group, Merseyside, UK) was tested on the isolated *P. aeruginosa* samples, as well as the control ATCC27853 strain by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Merck, Germany) based on Clinical Laboratory Standards Institute (CLSI) guidelines.

### Minimum Inhibitory Concentration of Antibiotics

Minimum inhibitory concentration (MIC) of different antibiotics were determined according to the guidelines of the CLSI by broth microdilution method. Distinct doses of imipenem, meropenem, cefepime, ampicillin, piperacillin/tazobactam, cefotaxime, ceftriaxone and ceftazidime (GLAXO England Co. and Himedia Co.) were added to the culture medium (Muller-Hinton broth (Merck, Germany)).

### Phenotypic detection of MBL

In this study, Meropenem and Meropenem + EDTA, Imipenem and Imipenem + EDTA discs were used to detect MBL producing *P. aeruginosa*. MBL detection was performed by Combination Disk Diffusion Test (CDDT).<sup>17</sup>

### Detection of bla<sub>IMP</sub> and bla<sub>VIM</sub> Genes by PCR Method

DNA templates were prepared by boiling method. The polymerase chain reaction (PCR) amplification for bla<sub>IMP</sub> and bla<sub>VIM</sub> were performed with primers VIM-F (5'-GTTTGGTTCGCATATCGCAAC-3') and VIM-R (5'-AATGCGCAGCACCAGGATAG-3') for bla<sub>VIM</sub> gene and primers IMP-F (5'-GAAGGCGTTTATGTTTCATAC-3') and IMP-R (5'-GTATGTTTCAAGAGTGATGC-3') for bla<sub>IMP</sub> gene under PCR conditions as reported previously.<sup>18</sup>

### Sequencing

The PCR purification kit (Bioneer Co., Korea) was used to purify PCR products and sequencing of forward strand was performed by the Bioneer company (Korea). The nucleotide sequences were analyzed with Chromas 1.45 and MEGA-4 softwares and BLAST in PubMed NCBI.

### Nucleotide sequence accession number

The nucleotide sequence data reported in this paper have been submitted to the GenBank sequence database and assigned accession no. JX648311.

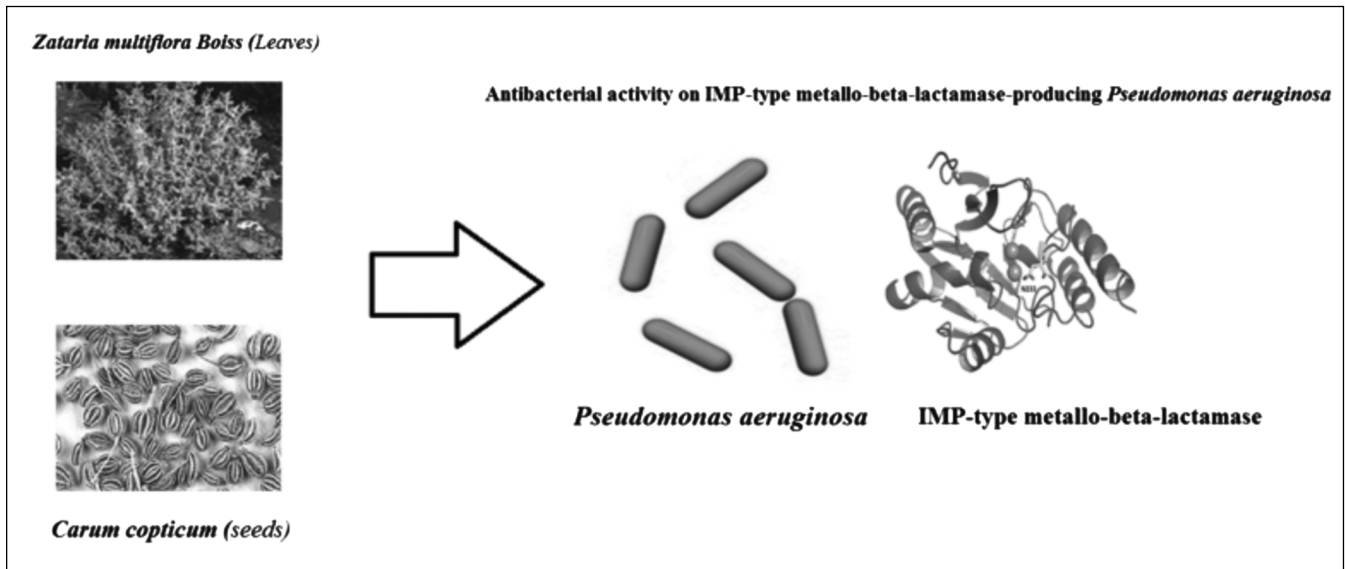


Fig. 1 - Antibacterial potency of *Carum copticum* and *Zataria multiflora* extracts against IMP-producing *P.aeruginosa* strains.

#### Plant material

##### Source, Collection and Identification

During 2012, leaves of *Zataria multiflora Boiss* and seeds of *Carum copticum* were collected from Fars province in Iran, during 2012 (Fig. 1). A voucher specimen was prepared and deposited at Shahid Beheshti University of Medical Sciences, Tehran, Iran.

##### Extraction Method

Leaves of the *Zataria multiflora Boiss* and seeds of *Carum copticum* plants (400gr) were dried at 25°C and then powdered using a mechanical grinder. 10gr of dried plant was soaked in 100ml methanol (96%, v/v), acetone (99%) and chloroform (99.4% purity) (Merck, Germany) for a period of 48 hours without heating. Each extract was first filtered through Whatman No. 1 filter paper to clarify and then through a 0.45 µm membrane filter. The filtrate was evaporated under reduced pressure in a vacuum evaporator and stored at 4°C. After drying, extracts were preserved in -20°C.

#### Determination of MIC and MBC values by broth Microdilution assay

The minimum inhibitory concentration (MIC) of the extracts was determined according to the method described by CLSI, 2012. *Zataria multiflora Boiss* and *Carum copticum* extracts were diluted with 10% DMSO to yield concentrations ranging from 25 to 0.19 mg/ml. Cation-adjusted Muller Hinton broth was used as broth medium. After shaking, 0.1ml of each extract was added to one well of 96-well microtiter plates. Microbial suspensions were ad-

justed to 0.5 MacFarland and diluted 1:10 to yield  $10^7$ CFU/ml and to each well, 0.005 ml of the bacterial inoculum were seeded. Control line with no bacterial inoculation and *P. aeruginosa* ATCC27853 was simultaneously maintained. Microplates were incubated aerobically at 37°C for 18-24 hours. The lowest concentration of the extracts that produced no visible bacterial growth was reported as the MIC. MBC values were determined using the first well showing no growth on media agar.

#### Statistical Analysis

The different effects of *Zataria multiflora Boiss* and *Carum copticum* extracts on *P. aeruginosa* were analyzed using one-way ANOVA via MINITAB Version 13 software. A p value <0.05 was considered significant.

#### Results

Out of the 100 *P.aeruginosa* isolates, 83 were resistant to Imipenem and Ceftazidime. The CDDT showed that among 83 Imipenem non-susceptible *P.aeruginosa* strains, 48 (57.9%) were metallo-beta-lactamase producers. All MBL-producing *P.aeruginosa* were resistant to Meropenem, Imipenem, Ceftazidime, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime and Carbenicillin; while 49% of isolates were resistant to Gentamicin, indicating that 100% of isolates were multi-drug resistant (MDR) (resistance to more than three antibiotics from different classes was defined as MDR). Minimum inhibitory concentration (MIC) of different antibiotics for IMP-producing *P.aeruginosa*

strains is shown in Table I. Using PCR method, 6 isolates were positive for bla(IMP) gene, while bla(VIM) gene was not detected. Sequencing of PCR products showed conserved region for the restriction sequence bla<sub>IMP</sub>-1 gene which was confirmed by BLAST. 48 (57.9%) patients were infected with MBL-producing *Pseudomonas* strains of whom 4 (8.3%) died. Antibacterial potency of *Carum copticum* and *Zataria multiflora* extracts against six IMP-producing *P.aeruginosa* strains (Fig. 1) were evaluated by microdilution method as described by CLSI. MICs and MBCs (mg/ml) results of *Carum copticum* and *Zataria multiflora* against IMP-producing *P.aeruginosa* strains were presented in Table II.

**Discussion**

The threat caused by *P.aeruginosa* in hospital-borne infections and the need for an effective antibiotic incited us to search for a potential anti-infective agent against this pathogen. It is assumed that herbal products could constitute a natural antibiotic for controlling and treating infection without risking the appearance of resistance strain.<sup>19</sup>

*P.aeruginosa* MBL producers are a major problem in medical centers because *P.aeruginosa* beta-lactamases-producers are capable of hydrolyzing almost all β-lactam antibiotics and are associated with high morbidity and

mortality.<sup>4</sup> In the past twenty years, *P.aeruginosa* was the most common bacteria in burn wards in Tehran, Iran.<sup>20</sup> *P.aeruginosa* isolates in our study showed resistance to almost all antibacterial agents. All MBL-producing *P.aeruginosa* were resistant to Meropenem, Imipenem, Ceftazidime, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime and Carbenicillin; while 49% of isolates were resistant to Gentamicin. In addition to enzymes mechanism, another mechanism such as Efflux pump or KPC enzymes with loss of the OprD (outer membrane porin) can also cause drug resistance.<sup>6</sup> Prevalence of drug resistance in burn wards has been reported from other parts of the world.<sup>4,9</sup> Compared to reports from the USA,<sup>21</sup> Spain,<sup>22</sup> Korea,<sup>23</sup> and India,<sup>24</sup> a higher prevalence of MBLs was found in Tehran probably due to different length of hospitalization, care conditions and antibiotics utilization protocols. Globally, as well as in Iran, the VIM beta-lactamase is the most dominant MBL type.<sup>25-27</sup> Previous research in Iran showed that 11.43% of *P.aeruginosa* isolates from Tehran and 19.51% from Ahwaz (in Iran) were VIM-type positive. However, in our study, IMP-1 producer *P.aeruginosa* was the dominant strain among the studied isolates, which is in concordance with other studies.<sup>28-30</sup> In this study, 42 isolates were negative for bla(VIM) and bla(IMP) genes by PCR method. However, resistance may be caused

**Table I-** Distribution of antibiotics MICs for IMP-producing *P.aeruginosa* strains

Antibiotics	MIC(µg/ml)					
	<i>P.a FSH2IMP</i>	<i>P.a FSH22IMP</i>	<i>P.a FSH28IMP</i>	<i>P.a FSH40IMP</i>	<i>P.a FSH42IMP</i>	<i>P.a FSH47IMP</i>
Imipenem	128	128	128	128	128	128
Meropenem	64	32	64	64	64	64
Cefepime	128	128	128	128	128	128
Ceftazidime	≥256	≥256	≥256	≥256	≥256	≥256
Cefotaxime	≥256	≥256	≥256	≥256	≥256	≥256
Ampicillin	≥256	≥256	≥256	≥256	≥256	≥256
Piperacillin/Tazobactam	256	256	256	256	256	256
Ceftriaxone	≥256	≥256	≥256	≥256	≥256	≥256

**Table II -** Frequency of minimum inhibitory concentration (MIC mg/ml) of *Carum copticum* and *Zataria multiflora* extracts for IMP-producing *P.aeruginosa* strains

Strain	<i>Carum copticum</i>						<i>Zataria multiflora</i>					
	Methanol		Acetone		Chloroform		Methanol		Acetone		Chloroform	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>P.a FSH2IMP</i>	6.25	6.25	12.5	12.5	25	25	6.25	12.5	6.25	12.5	6.25	12.5
<i>P.a FSH22IMP</i>	6.25	12.5	12.5	12.5	25	25	6.25	12.5	6.25	12.5	6.25	12.5
<i>P.a FSH28IMP</i>	6.25	6.25	12.5	12.5	25	25	6.25	6.25	6.25	6.25	6.25	6.25
<i>P.a FSH40IMP</i>	6.25	12.5	12.5	12.5	25	25	6.25	12.5	6.25	6.25	6.25	6.25
<i>P.a FSH42IMP</i>	6.25	6.25	12.5	12.5	25	25	6.25	6.25	6.25	6.25	6.25	6.25
<i>P.a FSH47IMP</i>	6.25	6.25	12.5	12.5	25	25	6.25	6.25	6.25	12.5	6.25	12.5
<i>P.aeruginosa ATCC27853</i>	1.56	3.125	6.25	12.5	6.25	12.5	1.56	3.125	1.56	3.125	3.125	6.25

by other genes such as AIM, SIM, KHM, SPM, GIM and NDM.<sup>6</sup>

In our study mortality rate due to infection with MBL-producing *Pseudomonas* strains was 4 (8.3%) and in Brazil the mortality rate of infection with MBL-producing *Pseudomonas* strains was 82.6%.<sup>31</sup> The genes encoding these enzymes spread easily on plasmids, causing nosocomial infections and outbreaks with mortality rates from 25% to 75%.<sup>32</sup> Only a few antibacterial drugs were effective on the *P.aeruginosa* MBL-producers that were isolated from Motahari hospital. Therefore there is a need to revise treatment protocols to prevent the spread of resistant genes. The antimicrobial activity of *Carum copticum* and *Zataria multiflora* have been demonstrated in many other studies but antibacterial effects of these plants against IMP-producing *P.aeruginosa* strains has not yet been reported.<sup>10-13</sup> The present study supports the idea that *Carum copticum* and *Zataria multiflora* extracts might be useful as antibacterial agents against IMP-producing *P.aeruginosa* strains. The acetic, methanolic and chloroformic extracts of *Zataria multiflora* and *Carum copticum* methanolic extract have promising MIC values against all IMP-producing *P.aeruginosa* in 6.25 mg/ml concentration. In this study, the antibacterial activity of these plants against IMP-producing *P.aeruginosa* strains was evaluated for the first

time. In 2010, Saei-Dehkordi and et al. reported that *P.aeruginosa* growth can be inhibited by the use of 2-8 mg/ml of *Zataria multiflora Boiss* essential oil.<sup>10</sup> Shariffar et al. have shown that essential oil and methanol extract of endemic *Zataria multiflora Boiss* has an inhibitory effect on *S. aureus*, *E. coli*, *K. pneumoniae*, *S.epidermidis*, *E. faecalis*, *B. subtilis*, *S.typhi*, *S.marcescens* and *S.flexneri*.<sup>11</sup> Furthermore, Mahboubi et al. have shown that *Staphylococcus* growth can be inhibited by 0.5-1 µl/ml of *Zataria multiflora Boiss* essential oils.<sup>12</sup>

### Conclusions

The incidence of MBL-producing *P.aeruginosa* in burn patients is very high. Therefore, detection of MBL-producing isolates is of great importance in identifying drug resistance patterns in *P.aeruginosa* and in preventing and controlling infections. The chloroformic, acetic and methanolic extract of *Zataria multiflora Boiss* and *Carum copticum* methanolic extract had a better effect on regular and clinical MBL-producing *P.aeruginosa* strains. Other evaluations considering the different effects of various herbal extracts as antibacterial agents, as well as *in vivo* examination of these extracts, are required to provide a natural, cost effective and strong alternative for the traditionally less effective antibiotics which are normally used.

**RÉSUMÉ.** La résistance aux carbapénèmes en raison de l'acquisition des métallo-bêta-lactamases (MBL) est considéré comme plus grave que d'autres mécanismes de résistance. Le but de cette étude était d'évaluer l'activité antibactérienne de *Zataria multiflora Boiss* et *Carum copticum* sur des souches de *Pseudomonas aeruginosa*. Cette étude expérimentale a été réalisée sur les patients brûlés hospitalisés au cours de 2011 et 2012. Les antibiotiques et des extraits des tests de sensibilité ont été réalisés par diffusion du disque et les méthodes de microdilution de bouillon. La détection des MBL a été réalisée par des tests basés sur l'utilisation de disques de combinaison. Les gènes bla(IMP) et bla(VIM) ont été détectés par des méthodes de PCR et séquençage. En utilisant la méthode de test de diffusion sur disque de combinaison, il a été constaté que, parmi les 83 souches de *Pseudomonas aeruginosa* résistant à l'imipénème, 48 (57,9%) étaient des producteurs de MBL. Méthodes de PCR et séquençage ont montré que ces isolats étaient positifs pour les gènes blaIMP-1, alors aucun n'était positif pour les gènes bla (VIM). Le taux de mortalité des patients hospitalisés avec une infection de *Pseudomonas* producteurs des MBL était de 4/48 (8,3%). Il a été montré que les extraits de *Zataria multiflora* et *Carum copticum* ont eu un effet antibactérien élevé sur des souches de *P. aeruginosa* réguliers et producteurs d'IMP dans 6,25 mg/ml de concentration. L'incidence du *P. aeruginosa* producteur des MBL chez les patients brûlés est très élevée. Dans notre étude, tous les isolats producteurs des MBL sont porteurs du gène blaIMP-1. Par conséquent, la détection des isolats qui produisent des MBL sont d'une grande importance dans l'identification des profils de résistance aux médicaments chez *P. aeruginosa* et dans la prévention et le contrôle des infections. Dans cette étude, il a été montré que des extraits de *Z. multiflora* et *C. copticum* ont des effets antibactériens élevés sur les souches de *P. aeruginosa* producteurs de β-lactamase.

**Mots-clés:** *P. aeruginosa*, metallo-β-lactamases, *Zataria multiflora Boiss*, *Carum copticum*

### BIBLIOGRAPHY

1. Dey D, Debnath S, Hazra S, Ghosh S, Ray R, Hazra B: Pomegranate pericarp extract enhances the antibacterial activity of ciprofloxacin against extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing Gram-negative bacilli. *Food Chem Toxicol*, 50: 4302-9, 2012.
2. Dharmalingam K, Tan BK, Mahmud MZ et al.: *Swietenia macrophylla* extract promotes the ability of *Caenorhabditis elegans* to survive *Pseudomonas aeruginosa* infection. *J Ethnopharmacol*, 139: 657-63, 2012.
3. Picao RC, Poirrel L, Gales AC, Nordmann P: Diversity of beta-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. *Antimicrob Agents Chemother*, 53: 3908-13, 2009.
4. Yousefi S, Farajnia S, Nahaei MR et al.: Detection of metallo-be-

- ta-lactamase-encoding genes among clinical isolates of *Pseudomonas aeruginosa* in the northwest of Iran. *Diagn Microbiol Infect Dis*, 68: 322-5 2009.
5. Poole K : *Pseudomonas aeruginosa*: Resistance to the max. *Front Microbiol*, 2: 65, 2011.
  6. Cornaglia G, Giamarellou H, Rossolini GM: Metallo-beta-lactamases: A last frontier for beta-lactams? *Lancet Infect Dis*, 11: 381-93, 2011.
  7. Miyajima Y, Hiramatsu K, Mizukami E et al.: In vitro and in vivo potency of polymyxin B against IMP-type metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *Int J Antimicrob Agents*, 32: 437-40, 2008.
  8. Khosravi Y, Tay ST, Vadivelu J: Analysis of integrons and associated gene cassettes of metallo-beta-lactamase-positive *Pseudomonas aeruginosa* in Malaysia. *J Med Microbiol*, 60: 988-94, 2011.
  9. Viedma E, Juan C, Villa J et al.: VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175 clone, Spain. *Emerg Infect Dis*, 18: 1235-41, 2012.
  10. Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F: Chemical composition of essential oils in *Zataria multiflora* Boiss from different parts of Iran and their radical scavenging and antimicrobial activity. *Food Chem Toxicol*, 48: 1562-7, 2010.
  11. Sharififar F, Moshafi MH, Mansouri SH, Khodashenas M, Khoshnoodi M: In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss. *Food control*, 18: 800-5, 2007.
  12. Mahboubi M, Bidgoli FG: Antistaphylococcal activity of *Zataria multiflora* essential oil and its synergy with vancomycin. *Phytomedicine: International journal of phytotherapy and phytopharmacology*, 17: 548-50, 2010.
  13. Misaghi A, Basti A.A: Effects of *Zataria multiflora* Boiss. essential oil and nisin on *Bacillus cereus* ATCC 11778. *Food Control*, 18: 1043-9, 2007.
  14. Boskabady MH, Jandaghi P, Kiani S, Hasanzadeh L: Antitussive effect of *Carum copticum* in guinea pigs. *J Ethnopharmacol*, 97: 79-82, 2005.
  15. Mohagheghzadeh A, Faridi P, Ghasemi Y: *Carum copticum* Benth essential oil chemotypes. *Food Chem Toxicol*, 100: 1217-9, 2007.
  16. Rezvani ME, Roohbakhsh A, Mosaddegh MH, Esmailidehaj M, Khaloobagheri F, Esmaeili H: Anticonvulsant and depressant effects of aqueous extracts of *Carum copticum* seeds in male rats. *Epilepsy Behav*, 22: 220-5, 2011.
  17. Galani I, Rekatsina PD, Hatzaki D, Plachouras D, Souli M, Giamarellou H: Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in Enterobacteriaceae. *J Antimicrob Chemother*, 61: 548-53, 2008.
  18. Khosravi AD, Mihani F: Detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infect Dis*, 60: 125-8, 2008.
  19. Bag A, Bhattacharyya SK, Pal NK, Chattopadhyay RR: In vitro antibacterial potential of *Eugenia jambolana* seed extracts against multidrug-resistant human bacterial pathogens. *Microbiol Res*, 167: 352-7, 2012.
  20. Estahbanati HK, Kashani PP, Ghanaatpisheh F: Frequency of *Pseudomonas aeruginosa* serotypes in burn wound infections and their resistance to antibiotics. *Burns*, 28: 340-8, 2002.
  21. Aboufaycal H, Sader HS, Rolston K et al.: blaVIM-2 and blaVIM-7 carbapenemase-producing *Pseudomonas aeruginosa* isolates detected in a tertiary care medical center in the United States: Report from the MYSTIC program. *J Clin Microbiol*, 45: 614-5, 2007.
  22. Riera E, Cabot G, Mulet X et al.: *Pseudomonas aeruginosa* carbapenem resistance mechanisms in Spain: Impact on the activity of imipenem, meropenem and doripenem. *J Antimicrob Chemother*, 66: 2022-7, 2011.
  23. Chin BS, Han SH, Choi SH et al.: The characteristics of metallo-beta-lactamase-producing gram-negative bacilli isolated from sputum and urine: a single center experience in Korea. *Yonsei Med J*, 52: 351-7, 2011.
  24. De AS, Kumar SH & Baveja SM: Prevalence of metallo-beta-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in intensive care areas in a tertiary care hospital. *Indian J Crit Care Med*, 14: 217-9, 2010.
  25. Correa A, Montealegre MC, Mojica MF et al.: First report of a *Pseudomonas aeruginosa* isolate coharboring KPC and VIM carbapenemases. *Antimicrob Agents Chemother*, 56: 5422-3, 2012.
  26. Jacobson RK, Minenza N, Nicol M, Bamford C : VIM-2 metallo-beta-lactamase-producing *Pseudomonas aeruginosa* causing an outbreak in South Africa. *J Antimicrob Chemother*, 67: 1797-8, 2012.
  27. Sardelic S, Bedenic B, Colinon-Dupuich C et al.: Infrequent finding of metallo-beta-lactamase VIM-2 in carbapenem-resistant *Pseudomonas aeruginosa* strains from Croatia. *Antimicrob Agents Chemother*, 56: 2746-9, 2012.
  28. Yoo JS, Yang JW, Kim HM et al.: Dissemination of genetically related IMP-6-producing multidrug-resistant *Pseudomonas aeruginosa* ST235 in South Korea. *Int J Antimicrob Agents*, 39: 300-4, 2012.
  29. Kitao T, Tada T, Tanaka M et al.: Emergence of a novel multidrug-resistant *Pseudomonas aeruginosa* strain producing IMP-type metallo-beta-lactamases and AAC(6')-Iae in Japan. *Int J Antimicrob Agents*, 39: 518-21, 2012.
  30. Jeannot K, Poirel L, Robert-Nicoud M, Cholley P, Nordmann P, Plesiat P: IMP-29, a novel IMP-type metallo-beta-lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*, 56: 2187-90, 2012.
  31. Marra AR, Pereira CA, Gales AC, Menezes LC, Cal RG, de Souza JM et al.: Bloodstream infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: epidemiology, microbiology, and clinical outcomes. *Antimicrobial agents and chemotherapy*, 50: 388-90, 2006.
  32. Maltezou HC: Metallo-beta-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? *International journal of antimicrobial agents*, 33: 405, 2009.

*Acknowledgments.* The authors would like to thank Dr. Mardaneh and Dr. Pourakbari from the Microbiology Department of Tehran University of Medical Sciences for providing IMP and VIM type positive isolates.

This paper was accepted on 2 June 2013.