

Determination of the prevalence of metallo- β -lactamases producing *Pseudomonas aeruginosa* strains from clinical samples by imipenem-EDTA combination disk method in Mottahari and Emam Khomainsi hospitals of Urmia

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Abstract

Introduction: *Pseudomonas aeruginosa* is one of the major bacterial nosocomial infections. Metallo- β -lactamases (MBLs) are one of the most important agents for resistance against carbapenem. Based on the role of carbapenem for *Pseudomonas* infection treatment, in this research clinical strains of MBL producing bacteria were studied.

Methods: Fifty *Pseudomonas aeruginosa* isolated from clinical samples were collected from patients that referred to the Emam Khomeini and Mottahari hospitals of Urmia. After bacterial confirmation with standard bacteriologic tests, bacterial sensitivity was assayed against ten common antibiotics by the method of disk diffusion test. Then imipenem-resistant strains were candidate for the identification of MBL production by imipenem-EDTA (Ethyl enediamine tetra acetic acid) combination disk test.

Results: Of all 50 isolated *Pseudomonas aeruginosa*, 36 isolates (72%) were resistant against imipenem. Obtained results from imipenem-EDTA combined disk test showed that 32.36 isolates (88.9%) had MBL.

Conclusion: Although *Pseudomonas aeruginosa* producing MBL are resistant against all of β -lactams antibiotics, but determining the strains that produce MBL can help physicians to select the suitable antibiotic for treatment and improve the prognosis of the infection due to this bacterium.

Keywords: Imipenem-EDTA combination disk test, Metallo- β -lactamase, *Pseudomonas aeruginosa*, Urmia

Introduction

Pseudomonads are gram-negative rods, aerobic and motile bacteria that are responsible for the outbreak of nosocomial infections in different parts of the world. One of the causes of serious infections, such as septicemia and nosocomial pneumonia, is carbapenem-resistant *Pseudomonas aeruginosa* strains. Some reports also show an increase in the number of these strains. Resistant to carbapenems is often associated with the production of metallo- β -lactamases (MBLs) (1). Bacterial infection of burn wounds is a major cause of disease and mortality in a burn unit and the most serious infections are caused by gram-negative organisms, which can increase the mortality by at least 50%. Of these, nosocomially-acquired *Pseudomonas aeruginosa* remains the major cause of mortality, particularly because of the emergence of multidrug-resis-

tant strains (2).

Carbapenems, especially imipenem is one of the most effective antibiotics against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is resistant to carbapenems by decreasing antibiotics absorption due to the lack of an outer membrane porin, as oprD, or exclusion from the cell by efflux pump and the production of MBL (2).

Reports show that MBL producing *P. aeruginosa* strains can increase mortality. A study by Bahar et al in martyr Motahari hospital, Tehran was burning, the death rate from *Pseudomonas* strains producing MBLs, 6.82% respectively, while the death rate from *Pseudomonas* strains lacked MBLs, 7.22% (2). Today, the emergence of antibiotic-resistant strains of *Pseudomonas aeruginosa* Vmvd lactamase as one of the challenges is Dcharsvkhtgy patients (3).



MBLs are not inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. MBL inhibitors in a laboratory environment include EDTA (Ethylenediaminetetraacetic acid), SMA (sodium mercaptoacetic acid), MPA (mercaptpropionic acid), and DPA (dipicolinic acid). Several different methods has been reported for identification of producing MBL strains that among these methods, use of these inhibitors is. Meanwhile, using of EDTA as a non toxic substance , easily accessible and ability of keep disks containing of EDTA to 16 weeks at 20°C are, adequate than other inhibitors (4,5). Several methods have been proposed to detect MBLs but an appropriate laboratory test method that has high sensitivity and specificity for the detection of the enzyme is crucial. These methods include DDST (double disk synergy test), E-test MBL, modified Hodge test (MHT), and pulsed-field gel electrophoresis (PFGE) (6-8).

Methods

Bacterial strains

This study was performed in Mottahari and Imam Khomeini hospitals in Urmia, from February 2013 to July 2014. Microbiological samples were obtained from clinical specimens, of which 50 isolates were identified as *P. aeruginosa*. These isolates were based on microscopic and biochemical tests such as on blood gram stain characteristics, oxidase test, catalase test, oxidative-fermentative test, growth on triple sugar iron (TSI) agar, growth on sulfide-indole-motility (SIM), growth on cetrimide agar and growth at 42. Then, Strains were preserved in trypticase soy broth (TSB) media (Merck, Germany) containing 20% (v/v) glycerol (9-11).

Antibiotic susceptibility tests

The susceptibility pattern of isolates for different antibiotics was tested using disk diffusion method (Kirby-Bauer) on Muller-Hinton agar plates (Merck, Germany) according to guidelines of Clinical and Laboratory Standard Institute (CLSI). The antimicrobial disks used were imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), carbenicillin (100 µg), tobramycin (10 µg), amikacin (30 µg), Ticarcilin (75 µg), gentamicin (10 µg), cefotaxime (30 µg), and ceftizoxime (30 µg) (provided by Mast-UK). *P. aeruginosa* ATCC 27853 was used as a control strain for susceptibility testing. Bacteria were inoculated into TSB, and incubated at 35°C for 2 to 4 hours until they reached the turbidity of a 0.5 McFarland standard. Then using a sterile swab were culture on Meller-Hinton agar plate, streaking in 3 directions over the entire agar surface. Finally antimicrobial disks were placed on the plate and incubated at 35°C for 18 to 24 hours. After incubation, measure the diameters of the zone of complete inhibition.

EDTA-IMP

Phenotypic detection of MBL production (EDTA-IMP) was carried out for imipenem-resistant strains by dis-

solving 186.1 g of disodium EDTA. 2H₂O in 1000 ml of distilled water, 0.5 mM EDTA solution was provided and adjusted to pH 8 by adding NaOH. Then, 930 µg of prepared solution was added to imipenem disk, and it was dried in an incubator. EDTA-imipenem disk as well as imipenem disk were placed in a plate containing Muller-Hinton agar and cultured *P. aeruginosa*. After 18 hours of incubation at 35°C, an organism was considered positive MBL if the inhibition zone diameter increased by 7 mm or more towards the IMP plus EDTA in comparison to IMP disk alone (12,13).

Results

In this study, 50 clinical isolates were identified as *P. aeruginosa* using the standard bio-chemical test. Based on the results, among 50 *P. aeruginosa* isolates, 36 (72%) lineages regarding phenotype were resistant to "imipenem" and among these 32 (88.9%) lineages were reproductions of β-lactamase (Figure 1). Table 1 shows, the sensitivity pattern and the resistance of *Pseudomonas aeruginosa* Isolates to 10 common and relevant antibiotics. As it can be seen from Table 1, the results from antibiogram using the Kirby-Bauer method based on the CLSI guidance and referring to the table related to the resistance, the average of sensitivity is reported. Results of phenotype of producing of *Pseudomonas aeruginosa* lineage MBL, using the combination disk diffusion test (CDDT), IMP-EDTA method showed that most resistance lineages to the imipenem were the reproduction of MBL (Figure 2).

Discussion

Carbapenems (such as imipenem, meropenem, dory penem and ortapenem) are important kinds of beta-lactam drugs which are resistant against the beta-lactamases, and are used in curing bacterial diseases in which they are able to hydrolyze penicillin's and cephalosporin's. According to being resistance to these antibiotics among bacterias especially *Pseudomonas aeruginosa* which has an important role in creating infectious diseases, a serious threat

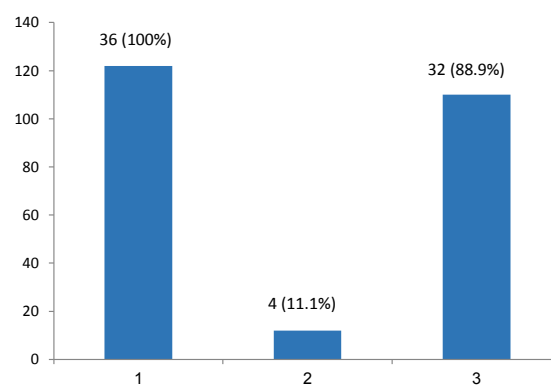


Figure 1. Prevalence determination of metallo-β-lactamases (MBLs) producing *Pseudomonas aeruginosa* strains by Imipenem-EDTA combination disk method. 3: positive MBLs; 2: Negative MBLs; 1: resistance against imipenem.

Table 1. Resistance pattern of *P. aeruginosa* strains

Antibiotics	Resistance	Intermediate	Sensitive	Percentage of resistance	Percentage of sensitivity
Imipenem (10 µg)	33	3	14	66	28
Meropenem (10 µg)	35	1	14	70	28
Ceftazidime (30 µg)	39	1	10	78	20
Carbenicillin (100 µg)	37	0	13	74	26
Tobramycin (10 µg)	41	1	8	82	16
Amikacin (30 µg)	38	1	11	76	22
Ticarcillin (75 µg)	39	0	11	78	22
Gentamicin (10 µg)	39	0	11	78	22
Cefotaxime (30 µg)	30	8	12	60	24
Ceftizoxime (30 µg)	42	3	5	84	10

**Figure 2.** MBL screening by CDDT. (A) Meropenem disc (10 µg). (B) Meropenem disc (10 µg) + 10 µl EDTA. (C) Imipenem disc (10 µg). (D) Imipenem disc (10 µg) + 10 µl EDTA.

in curing these infections is created. The appearance and obtaining MBLs among gram negative bacteria, especially those bacteria that has an important role in creating clinical infections, are important at least due to 2 reasons. Regarding epidemiologic, relevancy and increase of MBLs not only causes the resistance against carbapenems, it also is followed by the resistance against a lot of beta-lactam and non-beta-lactam antibiotics (14,15).

Beta-lactamases encoding genes are usually carried and encoded by versatile genetic elements. Hence, they can easily be transferred to sensitive lineages (16,17). Versatile genetic elements are parts of DNA which enter and exit from the genome that the most important ones are insertion sequence and plasmids. Most of bacteria such as *Pseudomonas* include plasmids that give them the property of being resistant against specific antibiotics. Recent studies shows, increase of resistance against these antibiotics especially by MBLs, are in the country. The analysis and study in 2007 on 120 *Pseudomonas aeruginosa* isolates from Scorch Department in Kerman SHAFI hospital has been done. None MBLs reproductive isolates, were reported. Also in research of Mihani et al (18) on *pseudomonas aeruginosa*

isolates, which is done in the Scorch Department of the hospital in Ahvaz city among 100 samples of clinical isolates, 42 samples (42%) had resistance against imipenem, which 8 samples (19.04%) were reported as MBLs reproductive. Also in the research which was done by Sadeghi et al (12) in 2012 in Arak, among the 108 studied isolates, 40 isolates (37%) were resistant against imipenem which 20 isolates (50%) were converted into positive regarding MBLs reproductive using disk diffusion method. In the comparison of the current studies and done studies in Iran, it was observed that the difference in the resistance pattern against imipenem is in the country, in which one reason is due to the prescribed antibiotics and the curing methods. In this study among 50 studied *Pseudomonas aeruginosa* isolates, 36 isolates (72%) were resistance against imipenem, which among them 32 isolates (88.9%) were MBLs reproductive, using the disk diffusion. This shows that these beta-lactamases reproductive lineages relevancy is getting higher that is under consideration. In a study which was done in Bangladesh in 2010, among 40 *Pseudomonas aeruginosa* isolates which were collected from patients, 23 isolates (57.5%) were resistant against imipenem, that among them 10 isolates (43%) were identified as beta-lactamases reproductive (19). In a study which was done in 2012 in Brazil, among 108 *Pseudomonas aeruginosa* isolates, 40 isolates (37%) were resistant against imipenem, in which 20 isolates (50%), regarding MBLs reproductive were changed into positive using disk diffusion IMP-EDTA (20). Fortunately in the comparison of the researches and investigations about the relevancy of MBLs in Iran, again it is revealed that, these kind of beta-lactamases is lower in Iran in compare of the other countries. However it is not beyond the expectations that the amount of usages of this remedy will increase considering the rising of these beta-lactamases reproductive isolates. The results of this study illustrate that the amount of *Pseudomonas aeruginosa* reproductive of MBLs relevancy in patients is high. Since, MBLs reproductive isolates can be resistant against all beta-lactam antibiotics, identifying resistant *Pseudomonas aeruginosa* against carbapenem,

which are able to produce MBLs is necessary and can help doctors in choosing a better remedy to cure the diseases which are infected by these kinds of infections.

Conclusion

The results of this study illustrates that the amount of *Pseudomonas aeruginosa* reproductive of MBLs relevancy in patients are high. Since, MBLs reproductive isolates can be resistant against all beta-lactam antibiotics, identifying resistant *Pseudomonas aeruginosa* against carbapenam, which are able to produce MBLs is necessary and can help the doctors in choosing a better remedy to cure the diseases which are infected by this kind of infections.

Ethical issues

The study was approved by the local ethic committee by Islamic Azad University, Urmia Branch.

Authors' contributions

The corresponding author acknowledges that each author has read and agrees with the information contained in the Author Role of the journal at the time of initial manuscript submission.

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