

Brief Communication

Detection of bla(IMP) and bla(VIM) metallo- β -lactamases genes among *Pseudomonas aeruginosa* strains

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Abstract: Acquired Metallo- β -Lactamases (MBLs) are emerging resistance determinants in *Pseudomonas aeruginosa* and other gram-negative bacteria. Using Combination Disk Diffusion test, it was found that among 83 imipenem non-susceptible *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 due to MBL-producing *Pseudomonas* infection was 4 (8.3%) among the hospitalized patients. Therefore, identification of drug resistance patterns in *P. aeruginosa* and detection of MBLs producing isolates are of great importance in the prevention and control of infections.

Keywords: *P. aeruginosa*, metallo- β -lactamases, antibiotics, mortality

Introduction

Burn patients are at risk for acquiring infection with *Pseudomonas aeruginosa* strains because of their body skin is destroyed and stopped immune system. *P. aeruginosa* is the common cause of nosocomial infections in patients and as an opportunist pathogen cause some infections such as pneumonia, septicemia, urinary tract infection, endocarditis, skin, ears and eyes infections and as a leading cause of morbidity and mortality among hospitalized burnt patients [1]. Since their first report in 1990s, metallo- β -lactamase (MBL)-producing bacteria have been detected in many parts of the world. The appearance of MBL enzymes and their spread among *P. aeruginosa* strains are matters of important concern with regard to the future of antibacterial chemotherapy [2]. Therefore, the aim of this study was detected of Verona imipenemase (VIM) and Imipenemase (IMP) metallo- β -lactamase genes on *P. aeruginosa* isolated from hospitalized burn patients in the Shahid Motahari Hospital, Tehran, Iran during the 2012 year.

Materials and methods

Between January to September 2012, from 448 burnt patients who had referred to Shahid Motahari Hospital, 100 isolates of *P. aeruginosa* were detected by laboratory conventional tests. *P. aeruginosa* ATCC27853 was used as a control strain. A disc diffusion test using antibiotics (Mast Group, Merseyside, UK) was performed according to the Clinical Laboratory Standards Institute (CLSI) guidelines [3] and MBL detection was performed by Combination Disk Diffusion Test (CDDT) [4]. DNA templates were prepared by boiling method and Polymerase chain reaction (PCR) amplification for blaIMP and blaVIM were performed with primers VIM-F (5'-GTTTGGTCGCATATCGCAAC-3') and VIM-R (5'-AATGCGCAGCACCAGGATAG-3') for bla_{VIM} gene and primers IMP-F (5'-GAAGGCGTTTATGTTTCATAC-3') and IMP-R (5'-GTATGTTTCAAGAGTGATGC-3') for bla_{IMP} gene under PCR conditions as described previously [5]. The PCR purification kit (Bioneer Co., Korea) was used to purify PCR products and sequencing of forward strand was performed by the Bioneer Company

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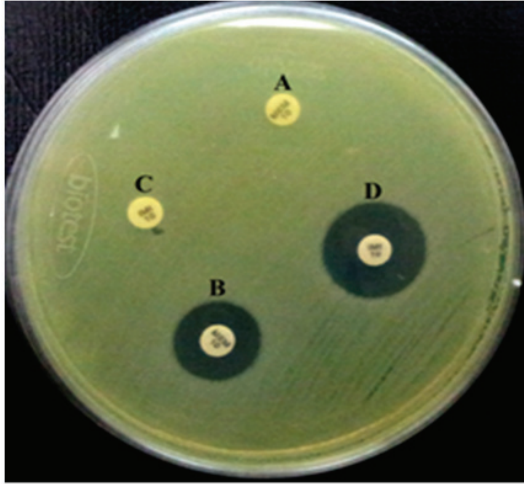


Figure 1. MBL screening by CDDT test showed that 48 (57.9%) of imipenem-nonsusceptible isolates were MBL positive. A MEM disc, B MEM 10µg+EDTA 10 µg disc, C IMI 10 µg disc and D IMI10µg+EDTA 10 µg disc.

(Korea). The nucleotide sequences were analyzed with Chromas 1.45 and MEGA-4 softwares and BLAST in NCBI and data reported in this paper have been submitted to the GenBank sequence database and assigned accession no. JX644173.

Results

Out of the 100 *P. aeruginosa* isolates, 83 (83%) were resistant to Imipenem. The CDDT showed that among the 83 imipenem non-susceptible *P. aeruginosa* strains, 48 (57.9%) were metallo-beta-lactamase producers (**Figure 1**). All MBL-producing *P. aeruginosa* were resistant to Meropenem, Imipenem, Ceftazidime, Cefotaxime, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime and Carbenicillin; while 49% of isolates were resistant to Gentamicin. Using PCR method, 6 isolates were positive for bla (IMP) gene, while bla(VIM) gene was not detected. Sequencing of PCR products showed bla_{IMP-1} gene which was confirmed by BLAST. 48 (57.9%) of patients were infected with MBL-producing *P. aeruginosa* strains and of whom 4 (8.3%) died.

Discussion

Recently, *P. aeruginosa* is known as most common bacteria in burn wards in Tehran, Iran. In this study, 48 (57.9%) of these strains were found to be MBL producers which were higher

than the study conducted by Mohammad Ali Bahar and et al. at the Shahid Motahari Hospital, Tehran, Iran during 2007-2008 years. Previous studies showed that 17.3% of *P. aeruginosa* isolates from Orumieh and Tabriz cities in northwest of Iran and 19.51% from Ahwaz (southwest of Iran) were VIM-type positive [6]. The mortality rate for MBL-producing *Pseudomonas* was 4 (8.3%) at Shahid Motahari Hospital during 2011-2012 years. The overall rate of mortality among patients infected with *P. aeruginosa* is high. Only a few antibacterial drugs were effective on the *P. aeruginosa* MBL-producers that were isolated from Shahid Motahari hospital. Therefore, control and treatment of these infections caused by the mentioned bacteria is difficult. So far, in Iranian studies on *P. aeruginosa*, the emphasis was on identification of Ambler class A and Ambler class D serine OXA [3] and also three reports on Ambler class B beta-lactamases [3, 5, 6].

In conclusion, we have shown that IMP-1 producing *P. aeruginosa* strains is an emerging threat in burn care parts and should be contained by implementation of timely identification and strict isolation methods.

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References

- [1] Tawfik AF, Shibl AM, Aljohi MA, Altammami MA, Al-Agamy MH. Distribution of Ambler class A, B and D beta-lactamases among *Pseudomonas aeruginosa* isolates. *Burns* 2012 Sep; 38: 855-60.

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- [2] Lee NY, Yan JJ, Lee HC, Liu KH, Huang ST, Ko WC. Clinical experiences of bacteremia caused by metallo-beta-lactamase-producing gram-negative organisms. *J Microbiol Immunol Infect* 2004 Dec; 37: 343-9.
- [3] Bahar MA, Jamali S, Samadikuchaksaraei A. Imipenem-resistant *Pseudomonas aeruginosa* strains carry metallo-beta-lactamase gene bla(VIM) in a level I Iranian burn hospital. *Burns* 2010 Sep; 36: 826-30.
- [4] 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* 2008 Mar; 61: 548-53.
- [5] Khosravi AD, Mihani F. Detection of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains isolated from burn patients in Ahwaz, Iran. *Diagn Microbiol Infect Dis* 2008 Jan; 60: 125-8.
- [6] Yousefi S, Farajnia S, Nahaei MR, Akhi MT, Ghotaslou R, Soroush MH, Naghili B, Jazani NH. Detection of metallo-beta-lactamase-encoding genes among clinical isolates of *Pseudomonas aeruginosa* in northwest of Iran. *Diagn Microbiol Infect Dis* 2010 Nov; 68: 322-5.