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

Fatemeh Fallah¹, Rebwar Shams Borhan², Ali Hashemi²

¹Pediatric Infectious Research Center, Mofid Children Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran; ²Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received January 30, 2013; Accepted March 7, 2013; Epub April 18, 2013; Published April 30, 2013

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 opportunistic 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 blaVIM gene and primers IMP-F (5′-GAAGGCCGTGATTTATGTTTACAT-3′) and IMP-R (5′-GTATGTTTCAAGAGTATGCAAC-3′) for blaIMP 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.
Detection of bla(IMP) and bla(VIM) metallo-β-lactamases

(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 Carbencillin; while 49% of isolates were resistant to Gentamycin. 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 B 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.

Acknowledgments

This work was financially supported by Dr. Karimi of Pediatric Infectious Research Center in Mofid Children Hospital and Microbiology department of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The authors thank Dr. Mardaneh and Dr. Pourakbari from Microbiology Department of Tehran University of Medical Sciences for providing the IMP and VIM type positive isolates.

Address correspondence to: Dr. Ali Hashemi, Department of Microbiology, Shahid Beheshti University of Medical Sciences, Koodakyar St, Tabnak Blvd, Yaman AV, Chamran Highway, Tehran, Iran. Phone: +98 21 23872556 or +989122947439; E-mail: hashemi1388@yahoo.com, ali.hashemi@sbmu.ac.ir

References

Detection of bla(IMP) and bla(VIM) metallo-β-lactamases


