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OCURRENCE OF *BLA*$_{SPM-1}$ AND *BLA*$_{IMP-1}$ GENES OF METALLO-β-LACTAMASES IN CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM THREE UNIVERSITARY HOSPITALS IN THE CITY OF PORTO ALEGRE, BRAZIL

Patrick Barcelos Gaspareto[1]; Andreza Francisco Martins[1]; Alexandre Prehn Zavascki [2]; Afonso Luis Barth[1,3] *

[1]Post-Graduation Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Rio Grande do Sul, RS, Brazil;
[2]Post-Graduation Program in Medical Sciences, Faculty of Medicine, Federal University of Rio Grande do Sul, RS, Brazil;
[3]Clinical Microbiology Unit, Clinical Hospital of Porto Alegre, Federal University of Rio Grande do Sul, RS, Brazil

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ABSTRACT

We described the ocurrence of metallo-β-lactamases (MBL) genes *bla*$_{SPM-1}$ and *bla*$_{IMP-1}$ in clinical isolates of *Pseudomonas aeruginosa* resistant to imipenem and/or ceftazidime obtained from three universitary hospitals in the city of Porto Alegre, Brazil. The MBL production was screened by phenotypic test and the genes were detected by PCR.

Key words: *Pseudomonas aeruginosa*; imipenem resistance; metallo-β-lactamase

The introduction of carbapenems into clinical practice heralded a new treatment option for serious bacterial infection (8). However, carbapenem resistance has now been observed in *Enterobacteriaceae* and in non-fermentative Gram-negative rods such as *Pseudomonas aeruginosa* and *Acinetobacter* spp. The common form of resistance is due to either lack of drug permeability (i.e. porin mutation and efflux pumps) and/or carbapenem-hydrolysing β-lactamase (8). The later included the metallo-β-lactamase (MBL), a group of clinically important enzymes because of their ability of hydrolyzing a broad range of β-lactams agents, including the carbapenems, and the absence of a clinically useful inhibitor (5). There are three main families of MBL reported around the world: IMP, VIM, and SPM. More recently, two other families have been reported: GIM and SIM (2,9). In Brazil, Gales *et al*, in 2003 reported the occurrence of a clone of *P. aeruginosa* producing SPM-1 in five Brazilian states (4). The aim of this study was to evaluate the presence of *bla*$_{SPM-1}$, *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ genes in 60 clinical isolates of *P.aeruginosa* resistant to imipenem and/or ceftazidime from three teaching Hospitals in the city of Porto Alegre, Brazil : Hospital São Lucas (HSL), Complexo Hospitalar Santa Casa (CHSC) and Hospital de Clínicas de Porto Alegre (HCPA). These isolates were obtained in 1998/99 (3 samples from HSL; 3 samples from CHSC) and in 2003/04 (14 samples from HCPA; 10 samples from HSL; 30 samples from CHSC). The production of MBL was screened by the disk approximation test using the mercaptopropionic acid (1). Oligonucleotide primers targeting conserved regions of *bla*$_{SPM-1}$, *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ genes (4,6,7) were used to determine the genetic basis of resistance by polymerase chain reaction (PCR) for phenotypic screen-positive isolates. The cycling parameters of PCR to amplify gene *bla*$_{SPM-1}$ were 95ºC for 5 min, followed by 30 cycles (95ºC for 1 min, 50ºC for 1min and 68ºC for 1 min). The PCR for gene *bla*$_{IMP-1}$ used cycling parameters as: 95ºC for 2 min, 94ºC for 2 min followed by 33 cycles (94ºC for 1 min, 60ºC for 1 min and 72ºC for 1 min). The PCR for gene *bla*$_{VIM-2}$ were: 94ºC for 3 min followed by 35 cycles (94ºC for 1 min, 61ºC for 1 min and 72ºC for 1 min) with a final extension of 72ºC for 7 min. *P.aeruginosa* strains harbouring the *bla*$_{SPM-1}$, *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ MBL genes were used as a positive control. The amplification of DNA fragments of 650 bp, 580bp and 800bp confirmed the presence of *bla*$_{SPM-1}$, *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ genes respectively. The phenotypic test of disk approximation showed that *P. aeruginosa* was positive for MBL in 11 isolates from

*Corresponding Author. Mailing address: Hospital de Clínicas de Porto Alegre - Unidade de Microbiologia e Biologia Molecular - Serviço de Patologia Clínica - Rua Ramiro Barcelos, 2350. Cep 90035-003 - Porto Alegre, RS - Brasil. E-mail: albarth@hcpa.ufrgs.br

HSL 84,61% (11/13), in 24 isolates from CHSC 72,72% (24/33), and in 14 isolates HCPA 100% (14/14). The PCR procedure identified the bla_{SPM-1} gene in nine samples from HSL. A total of 16 clinical isolates (67,0%) from CHSC and 5 clinical isolates (35,71%) from HCPA also presented the gene bla_{SPM-1}. The gene bla_{IMP-1} was positive in 2 clinical isolates (8,33%) from CHSC and 3 clinical isolates (21,43%) from HCPA. The gene bla_{VIM-2} was not detected in the clinical isolates evaluated in this study. A total of six isolates obtained from CHSC, two isolates obtained from HSL, and six isolates obtained from HCPA produce no PCR amplification products with any of the primers used although they were characterized as MBL producer according to the phenotypic test. The dissemination of metallo-β-lactamase has been a concerning problem around the world triggering surveillance programs. A special focus on *P. aeruginosa*, a common pathogen in the hospital environmental which leads to serious infection mainly in immunocompromised patients, is necessary because this species is the most common non-fermentative Gram-negative rod harboring the MBL gene (6). Sader *et al*, 2005 (7) reported in one medical center in the city of São Paulo the ocurrence of IMP, VIM and SPM metallo-β-lactamases in *P. aeruginosa* recovery between 2000-2001. In their study, from a total of 36 isolates MBL positive, 20 isolates (55,60%) were bla_{SPM-1}, 11 (30,60%) were bla_{VIM-2} and 3 (8,30%) were bla_{IMP-1}. They also found that two isolates did not produce PCR amplification products with any of the primers employed. In our study, although the prevalence of the gene bla_{SPM-1} was different (73,0% vs. 55,60%), this gene was also found as the most common among *P. aeruginosa* MBL positive. In another hand, we did not find bla_{VIM-2} gene in our study. The fourteen isolates that produced MBL but were negative in PCR product should be tested with primers for other MBL genes and/or undergo isoelectric focusing (IFE) to discover the determinant of resistance. The clinical isolates from HSL positive to bla_{SPM-1} were typed by Pulsed Field Gel Electrophoresis (PFGE) and showed the presence of a single clone with four related subtypes characterizing an outbreak (10). Therefore, it is necessary to evaluate the profile of DNA macrorrestrition of clinical isolates from CHSC and HCPA to establish whether the occurrence of MBL positive *P. aeruginosa* in these hospitals is also due to a clonal dissemination. This data will be of importance for the hospital infection committee to adopt measures to control the dissemination of MBL positive *P. aeruginosa*.

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RESUMO

Ocorrência dos genes de metalo-β-lactamases bla_{SPM-1} e bla_{IMP-1} em isolados clínicos de *Pseudomonas aeruginosa* de três hospitais universitários da cidade de Porto Alegre, Brasil

Descrevemos a ocorrência dos genes de metalo-β-lactamases (MBL) bla_{SPM-1} e bla_{IMP-1} em isolados clínicos de *Pseudomonas aeruginosa* resistentes ao imipenem e/ou ceftazidima obtidos em três hospitais universitários de Porto Alegre, Brasil. A produção de MBL foi observada através de técnica fenotípica e os genes foram detectados pelo método de PCR.

Palavras chave: *Pseudomonas aeruginosa*, resistênica a imipenem, metalo-β-lactamase

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