

Case Report

Identification of São Paulo metallo-beta-lactamase-1-producing *Pseudomonas aeruginosa* in the Central-West region of Brazil: a case study

Wirlaine Glauce Maciel^[1], Kesia Esther da Silva^[1], José Victor Bortolotto Bampi^[1], Graciela Mendonça dos Santos Bet^{[1],[2]}, Ana Carolina Ramos^[3], Ana Cristina Gales^[3] and Simone Simionatto^[1]

[1]. Laboratório de Pesquisa em Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brasil.
[2]. Hospital Universitário de Dourados, Universidade Federal da Grande Dourados, Dourados, MS, Brasil. [3]. Laboratório Alerta, Disciplina de Infectologia, Departamento de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil.

Abstract:

Metallo-beta-lactamase production is an important mechanism for carbapenem resistance of *Pseudomonas aeruginosa*, which represents an emerging public health challenge. We report the case of a patient admitted to an intensive care unit, with sepsis caused by multidrug-resistant São Paulo Metallo-beta-lactamase-1-producing *P. aeruginosa*. This is the first case of infection by this pathogenic strain in the State of Mato Grosso do Sul, Brazil. Thus, infection control measures are required for preventing future spread and outbreaks.

Keywords: *Pseudomonas aeruginosa*. Metallo-beta-lactamase. SPM.

INTRODUCTION

Pseudomonas aeruginosa is a leading cause of nosocomial infections, particularly in intensive care units (ICUs). The prevalence of carbapenem resistance in these bacteria has been increasing worldwide, especially in Brazil¹. The high frequency of genetic mutations that confer antibiotic resistance to these pathogens is a major concern in hospitals worldwide. The detection of multiple antimicrobial resistance determinants in these microorganisms is increasing. The production of metallo-beta-lactamase (MβL) by *P. aeruginosa* strains is considered one of the most important factors conferring resistance to β-lactam antibiotics, including carbapenems².

Several types of MβL have been identified among *P. aeruginosa* strains. Production of São Paulo metallo-beta-lactamase (SPM) has been associated with broad-spectrum β-lactam resistance, including carbapenem resistance, and has been identified as the predominant MβL in Brazil^{1,3}. SPM-1 was first detected and reported in 2001 in São Paulo, Brazil¹. Since then, SPM-1-producing *P. aeruginosa* strains have been reported in different regions of Brazil^{3,4}. However, data are

lacking regarding the clinical features of SPM-1-related drug resistance in the Central Western Brazilian State of Mato Grosso do Sul. Here, we report a multidrug-resistant *P. aeruginosa* strain producing SPM-1, which was isolated from a patient hospitalized in the ICU of a teaching hospital in the City of Dourados, Mato Grosso do Sul. The rapid identification of multidrug resistance is essential to prevent the spread of these microorganisms in hospitals, and to establish adequate therapies against this infection⁵.

CASE REPORT

In November 2014, a 57-year-old man was admitted to the ICU of a teaching hospital in the City of Dourados, Mato Grosso do Sul, a Central Western Brazilian state. The patient had been hospitalized for 6 days in another health facility where, owing to Fournier gangrene, he underwent a colostomy procedure and surgical debridement. The patient was admitted to our institution because of hemiplegia on the left side, which was caused by a previous stroke. He also displayed a low level of consciousness (Glasgow coma score of 6) and had a history of hypertension and diabetes. He was sedated and placed on mechanical ventilation via tracheostomy. On the day of admission, he underwent dialysis because of acute renal failure with suspicion of integument and urinary sepsis, which presented as lower limb edema and the discreet presence of debridement in the expansion region of the scrotum. He underwent procedures to introduce several invasive devices such as a central venous catheters, colostomy bag and urethral.

Corresponding author: Dra. Simone Simionatto.
e-mail: simonesimionatto@ufgd.edu.br

Received 6 July 2016

Accepted 8 September 2016

After 34 days of hospitalization, *P. aeruginosa* was isolated from urine culture samples. Intravenous treatment, which included teicoplanin (600mg) once a day and meropenem (1,000mg) twice a day, was administered for 26 days. On the 21st and 39th days of hospitalization, the patient experienced cardiopulmonary arrest without electrical activity or pulse; however, the medical staff managed to revive him. After 49 days of hospitalization, his condition had progressively worsened and *P. aeruginosa* was isolated from another urine sample. New antibiotic therapy was started with meropenem (500mg) and tigecycline (50mg) twice a day for 19 days. On the 60th day, the patient collapsed and experienced two heart attacks that progressed to death. The cause of death was reported as cardiorespiratory arrest aggravated by sepsis.

The *P. aeruginosa* strains were identified using the automated microbial testing system VITEK® 2 (bioMérieux, Marcy-l'Étoile, France) and confirmed by performing matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT spectrometer (Bruker Daltonics, Billerica, MA, USA)⁶. The minimal inhibitory concentrations (MICs) were determined using broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines⁷, except for tigecycline MICs, which were interpreted using the Food and Drug Administration guidelines⁸. *P. aeruginosa* showed sensitivity only to polymyxin B (MIC₅₀ 0.5µg/mL), colistin (MIC₅₀ 4µg/mL) and tigecycline (MIC₅₀ 0.5µg/mL). Two *P. aeruginosa* strains isolated had the same antimicrobial resistance profile.

Preliminary screening for the presence of carbapenemases was performed using the modified Hodge test according to CLSI guidelines⁷ and by ertapenem hydrolysis using MALDI-TOF MS⁹. The presence of genes encoding β-lactamase (*bla*_{IMP-1}, *bla*_{KPC-2}, *bla*_{NDM-1}, *bla*_{VIM-1}, *bla*_{OXA-48}, *bla*_{GES-1}, and *bla*_{SPM-1}) was detected using polymerase chain reaction (PCR), followed by sequencing using specific primers as previously described⁶. The *bla*_{SPM-1} gene was present in the multidrug-resistant *P. aeruginosa* strains. The predicted protein sequence was analyzed using the Lasergene Software Package (DNASTAR, Madison, WI, USA) and compared to the sequences deposited in GenBank. The presence of *bla*_{IMP-1}, *bla*_{NDM-1}, *bla*_{VIM-1}, *bla*_{OXA-48}, and *bla*_{GES-1} could not be confirmed.

Ethical considerations

This study was conducted with the approval of the Research Ethics Committee from the *Universidade Federal da Grande Dourados* (no. 877.292/2014) and in accordance with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. The patient involved in the study provided written informed consent prior to participation.

DISCUSSION

The spread of carbapenemase-producing *P. aeruginosa* strains is alarming, because this species is a main source of hospital-acquired infections in critically ill patients, and is known for its ability to transfer drug resistance genes². The

dissemination of carbapenemase-producing strains is of great concern to public health services in Brazil. Several outbreaks and sporadic cases of SPM-1-producing strains, which spread rapidly, have been reported in Brazil^{10,11}. However, to our knowledge, there have been no reports of SPM-1-producing *P. aeruginosa* strains in the State of Mato Grosso do Sul. Here, we report a case of a patient who died from uncontrolled infection complicated by sepsis and a urinary tract infection, which was originally caused by a multidrug-resistant SPM-1-producing *P. aeruginosa* strain. The patient had not traveled to any other state in which these pathogens have been previously reported. However, he was exposed to risk factors associated with acquisition of MβL-producing bacteria, such as an ICU stay, extended hospitalization, history of comorbidity and the use of invasive devices^{9,11}. This patient was not colonized at admission. His clinical history showed previous hospitalizations and surgical procedures before our initial culture was collected. However, this study was not able to identify the introduction of this strain into our hospital. Phenotypic test results were concordant with the detection of carbapenemase, and PCR and deoxyribonucleic acid (DNA) sequencing confirmed SPM-1-producing *P. aeruginosa*.

The patient died after administration of two different antimicrobial therapies. The initial treatment consisted of a combination of teicoplanin and meropenem. However, this inappropriate therapy may have contributed to the worsening of his clinical condition and the evolution of sepsis. *In vitro* test results suggested that polymyxin B and colistin represent alternative therapies. Although these drugs may be associated with severe nephrotoxicity and there is a paucity of data on clinical outcomes, they are still used as the last therapeutic choice against *P. aeruginosa* infections¹².

In this study, we confirmed the presence of SPM-1-producing *P. aeruginosa* in Mato Grosso do Sul, a Central Western Brazilian state. This report highlights the potential emergence of these strains, and demonstrates the need for strategies to improve infection control measures that prevent an increase in these nosocomial infections. The rapid identification of resistance profiles of carbapenemase-producing strains is crucial for timely implementation of appropriate antimicrobial therapy.

Conflict of interest

The authors declare that there are no conflicts of interest.

Financial support

This work was partially supported by the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq Grant 480949/2013-1) and the *Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul* (FUNDECT Grant 05/2011 and 04/2012). W.G.M. and K.E.S. received a scholarship from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES). A.C.G. is a researcher from the CNPq, Ministry of Science and Technology, Brazil (Process number: 307816/2009-5).

REFERENCES

1. Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metallo- β -lactamase-producing *Pseudomonas aeruginosa*. *Infect Chemother*. 2015;47(2):81-97.
2. Silva LV, Galdino ACM, Nunes APF, Santos KRN, Moreira BN, Cacci LC, et al. Virulence attributes in Brazilian clinical isolates of *Pseudomonas aeruginosa*. *Int J Med Microbiol*. 2014;304(8):990-1000.
3. Costa LMA, Fleming MECK, Paula GR, Teixeira LA, Mondino PJJ, Mondino SSB, et al. Production of metallo- β -lactamase among *Pseudomonas aeruginosa* strains isolated in the State of Sergipe, Brazil. *Rev Soc Bras Med Trop*. 2015;48(2):212-15.
4. Labarca JA, Salles MJC, Seas C, Guzmán-Blanco M. Carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the nosocomial setting in Latin America. *Crit Rev Microbiol*. 2016;42(2):276-92.
5. Pobiega M, Maciag J, Chmielarczyk A, Romaniszyn D, Pormoska-Wesolowska M, Ziolkowski G, et al. Molecular characterization of carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from patients with urinary tract infections in Southern Poland. *Diagn Microbiol Infect Dis*. 2015;83(3):295-7.
6. Fehlberg LCC, Nogueira KS, Silva RC, Nicolleti AG, Palmeiro JK, Gales AC, et al. Detection of PER-2-producing *Enterobacter cloacae* in a Brazilian liver transplantation unit. *Antimicrob Agents Chemother*. 2014;58(3):1831-2.
7. Clinical and Laboratory Standards Institute. CLSI M100-S26 Performance Standards for Antimicrobial Susceptibility Testing - Twenty-Sixth Informational Supplement. CLSI document M100-S26. Wayne, PA: Clinical and Laboratory Standards Institute, 2016.
8. Pankey GA. Tigecycline. *J Antimicrob Chemother*. 2005;56(3):470-80.
9. Rizek C, Fu L, dos Santos LC, Leite G, Ramos J, Rossi F, et al. Characterization of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates, carrying multiple genes coding for this antibiotic resistance. *Ann Clin Microbiol Antimicrob*. 2014;13:43.
10. Picão RC, Poirel L, Gales AC, Nordmann P. Diversity of beta-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. *Antimicrob Agents Chemother*. 2009;53:3908-13.
11. Jager P, Chirwa P, Naidoo S, Perovic O, Thomas J. Nosocomial outbreak of New Delhi metallo- β -lactamase-1-producing Gram-negative bacteria in South Africa: a case-control study. *Plos One*. 2015;10(4):e-0123337.
12. Abdeiraouf K, Braggs KH, Yin T, Truong LD, Hu M, Tam VH. Characterization of polymyxin B-induced nephrotoxicity: implications for dosing regimen design. *Antimicrob Agents Chemother*. 2012;56(9):4625-9.