IMP-16 in *Pseudomonas putida* and *Pseudomonas stutzeri*: potential reservoirs of multidrug resistance

Production of metallo- β -lactamases (MBLs) is one of the leading carbapenem resistance mechanisms among Gram-negative aerobes. The IMP- and VIM-type enzymes are considered the most widespread as they have been reported from several continents. These enzymes are very often encoded by integron-borne genes with several allelic variants known for each type (Queenan & Bush, 2007). While SPM is the most common MBL type in Brazil and a *Pseudomonas aeruginosa* epidemic clone has been detected in several Brazilian cities (Gales *et al.*, 2003; Carvalho *et al.*, 2006), IMP and VIM variants have also been described in some isolates of *P. aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* (Sader *et al.*, 2005; Penteado *et al.*, 2009). In this study, we report the presence of MBL IMP-16 in carbapenem-resistant *Pseudomonas putida* and *Pseudomonas stutzeri* isolates recovered from two children who attended two different hospitals in Rio de Janeiro, Brazil.

P. putida and *P. stutzeri* are environmental species reported as a rare cause of clinical infection. Infections caused by these agents have been described in immunosuppressed hosts or patients with invasive medical devices (Yee-Guardino *et al.*, 2006; Aumeran *et al.*, 2007). They should be considered pathogenic when isolated from pure culture. Unlike *P. aeruginosa*, they are usually susceptible to a variety of antibiotics (Sader & Jones, 2005).

P. stutzeri CCBH 4919 was isolated in March 2009 from cerebrospinal fluid (CSF) of a 7-year-old female patient in the intensive care unit (ICU) of the Prontobaby Hospital da Criança, a private paediatric hospital. The patient had a brain tumour and was admitted for surgery and insertion of external ventricular derivation in February 2009. Complications arose when she developed meningitis 23 days post-surgery. The patient initially received intravenous

(i.v.) meropenem and amikacin. Intrathecal amikacin doses (5 mg per day) were initiated 9 days after i.v. antibiotic therapy was prescribed due to persistent clinical symptoms and recurrent isolations of *P. stutzeri* in the CSF. Intrathecal doses were increased to 7.5 mg per day and the child received a total of 27 days of intrathecal and i.v. amikacin, 20 days of meropenem and 14 days of ciprofloxacin, with three consecutive negative CSF cultures. No side effects were attributed to intrathecal therapy. The child was discharged about 2.5 months after admission on 18 April 2009 with no neurological sequelae.

Few cases involving *P. stutzeri* in the central nervous system have been described in the English literature. One case occurred in a man infected with HIV who underwent antimycobacterial therapy for tuberculous lymphadenitis and whose CSF grew *P. stutzeri* after he presented with symptoms of meningitis (Roig *et al.*, 1996). Another case was in a child who had a hospital-acquired *P. stutzeri* brain abscess after subdural grid implantation before surgery for refractory epilepsy (Yee-Guardino *et al.*, 2006).

P. putida CCBH 4308 was recovered in June 2008 from blood of a 2-month-old female patient with interatrial communication hospitalized due to bronchiolitis in the paediatric ICU of Hospital dos Servidores do Estado, a tertiary public hospital. The patient responded clinically to ampicillin and gentamicin (i.v.) for 10 days and was discharged 12 days after *P. putida* isolation.

Species identification was realized by the API 20NE system (bioMérieux) and confirmed by 16S rRNA gene sequencing. The MICs of ceftazidime, cefotaxime, imipenem, meropenem, gentamicin, amikacin and polymyxin B were obtained using Etest (AB Biodisk). *P. putida* CCBH 4308 and *P. stutzeri* CCBH 4919 were

resistant to all tested β -lactams and susceptible only to amikacin, gentamicin and polymyxin B (Table 1). The β -lactam resistance pattern was unusual for members of these species (Sader & Jones, 2005). In particular, resistance to carbapenems suggests the possibility of carbapenemase production or another acquired resistance mechanism. In fact, MBL production was detected in both isolates using a ceftazidime–2-mercaptopyrroline acid double-disc synergy test and imipenem–EDTA combined-disc test. The isolates showed PCR-positive results by using *bla*_{IMP}-like primers; and amplicon sequencing revealed 100% sequence identity with *bla*_{IMP-16}. *P. putida* and *P. stutzeri* isolates were positive for the class 1 integron (Lévesque *et al.*, 1995) with amplicon lengths of about 2.3 kb and 1 kb, respectively, corresponding to the size of the variable region. DNA sequencing of the variable region revealed that the *P. stutzeri* integron carried *bla*_{IMP-16} only, and the *P. putida* integron carried three gene cassettes (*aacA7*, *aacA4* and *aadA1*) that confer resistance to aminoglycosides. However, the *bla*_{IMP-16} gene of *P. putida* was not located in this mobile element. The possibility exists that this gene is located on another undetected mobile element.

The presence of plasmid DNA was not observed in the *P. putida* isolate using a Midi preparation of plasmid DNA by alkaline lysis with SDS, as described by Sambrook & Russell (2001). The *P. stutzeri* isolate harboured a unique plasmid estimated to be about 2.3 kb in size. Southern blot hybridizations with digoxigenin-labelled *bla*_{IMP-16} generated by use of the PCR DIG detection system (Roche Diagnostics) were performed to determine the location of the MBL gene. The *bla*_{IMP-16} probe did not hybridize to plasmid extracts from *P. stutzeri*, suggesting that this gene was carried on the chromosome in both isolates.

Table 1. Antimicrobial susceptibility of IMP-16-producing *Pseudomonas putida* CCBH 4308 and *Pseudomonas stutzeri* CCBH 4919

Antibiotic	MIC (mg l ⁻¹)*	
	<i>P. putida</i> CCBH 4308	<i>P. stutzeri</i> CCBH 4919
Cefotaxime	>256	>256
Ceftazidime	>256	>256
Imipenem	>32	>32
Meropenem	>32	>32
Gentamicin	4	3
Amikacin	2	3
Polymyxin B	1.5	0.5

*Etest MIC.

An IMP-16 variant has been described before in a *P. aeruginosa* strain isolated from a 60-year-old man in a hospital in Brasília city, Brazil (Mendes *et al.*, 2004). The *bla*_{IMP-16} gene was found in a class 1 integron with a 2.9 kb variable region located on the chromosome of *P. aeruginosa*. To our knowledge, *bla*_{IMP-16} has only been described in the literature in this index strain. Our report is the first description of IMP-16 production in *P. stutzeri* and *P. putida* isolates.

Little is known about chromosomal and transferable carbapenem resistance in environmental *Pseudomonas* species, although MBLs have been reported particularly in *P. putida* (Juan *et al.*, 2010). In *P. stutzeri*, this is the second description of an IMP-type MBL. The first description was in Taiwan (Lee *et al.*, 2004). Our findings suggest that the environmental organisms acquired the MBL genes probably under selective pressure of antibiotic exposure in the hospital environment, and highlight the threat as a potential reservoir of MBLs and other resistance genes, corroborating what has been described previously (Juan *et al.*, 2010). Hence, hospital surveillance for MBL-producing isolates in environmental reservoirs is warranted.

Acknowledgements

We are grateful to the Genomic Platform/DNA sequencing PDTIS/FIOCRUZ (RPT01A) workers for DNA sequencing. This work was supported by grants from FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro) and the Oswaldo Cruz Institute/

FIOCRUZ. This report was presented in part at the 49th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, USA, 2009.

Ana P. D'A. Carvalho-Assef,¹
Marisa Z. R. Gomes,¹ André R. A. Silva,²
Lúcia Werneck,² Caio A. S. Rodrigues,³
Maria J. Souza³ and Marise D. Asensi¹

¹Nosocomial Infection Research Laboratory, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil

²Hospital Infection Control Committee, Prontobaby Hospital da Criança, Rio de Janeiro, RJ, Brazil

³Microbiology Laboratory, Hospital dos Servidores do Estado, Rio de Janeiro, RJ, Brazil

Correspondence: Ana P. D'A. Carvalho-Assef
(anapdca@ioc.fiocruz.br)

Aumeran, C., Paillard, C., Robin, F., Kanold, J., Baud, O., Bonnet, R., Souweine, B. & Traore, O. (2007). *Pseudomonas aeruginosa* and *Pseudomonas putida* outbreak associated with contaminated water outlets in an oncohaematology paediatric unit. *J Hosp Infect* 65, 47–53.

Carvalho, A. P. D'A., Albano, R. M., Oliveira, D. N., Cidade, D. A. P., Teixeira, L. M. & Marques, E. A. (2006). Characterization of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM-1 metallo- β -lactamase in a hospital located in Rio de Janeiro, Brazil. *Microb Drug Resist* 12, 103–108.

Gales, A. C., Menezes, L. C., Silbert, S. & Sader, H. S. (2003). Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM

metallo- β -lactamase. *J Antimicrob Chemother* 52, 699–702.

Juan, C., Zamorano, L., Mena, A., Alberti, S., Pérez, J. L. & Oliver, A. (2010). Metallo- β -lactamase-producing *Pseudomonas putida* as a reservoir of multidrug resistance elements that can be transferred to successful *Pseudomonas aeruginosa* clones. *J Antimicrob Chemother* 65, 474–478.

Lee, N. Y., Yan, J. J., Lee, H. C., Liu, K. H., Huang, S. T. & Ko, W. C. (2004). Clinical experiences of bacteremia caused by metallo- β -lactamase-producing gram-negative organisms. *J Microbiol Immunol Infect* 37, 343–349.

Lévesque, C., Piché, L., Larose, C. & Roy, P. H. (1995). PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 39, 185–191.

Mendes, R. E., Toleman, M. A., Ribeiro, J., Sader, H. S., Jones, R. N. & Walsh, T. R. (2004). Integron carrying a novel metallo- β -lactamase gene, *bla*_{IMP-16}, and a fused form of aminoglycoside-resistant gene *aac(6')-30/aac(6')-Ib*: report from the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother* 48, 4693–4702.

Penteado, A. P., Castanheira, M., Pignatari, A. C. C., Guimarães, T., Mamizukac, E. M. & Gales, A. C. (2009). Diagnostic dissemination of *bla*_{IMP-1}-carrying integron In86 among *Klebsiella pneumoniae* isolates harboring a new trimethoprim resistance gene *dftr23*. *Diagn Microbiol Infect Dis* 63, 87–91.

Queenan, A. M. & Bush, K. (2007). Carbapenemases: the versatile β -lactamases. *Clin Microbiol Rev* 20, 440–458.

Roig, P., Orti, A. & Navarro, V. (1996). Meningitis due to *Pseudomonas stutzeri* in a patient infected with human immunodeficiency virus. *Clin Infect Dis* 22, 587–588.

Sader, H. S. & Jones, R. N. (2005). Antimicrobial susceptibility of uncommonly isolated non-enteric Gram-negative bacilli. *Int J Antimicrob Agents* 25, 95–109.

Sader, H. S., Castanheira, M., Mendes, R. E., Toleman, M., Walsh, T. R. & Jones, R. N. (2005). Dissemination and diversity of metallo- β -lactamases in Latin America: report from the SENTRY Antimicrobial Surveillance Program. *Int J Antimicrob Agents* 25, 57–61.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Yee-Guardino, S., Danziger-Isakov, L., Knouse, M., Bingaman, W., Sabella, C. & Goldfarb, J. (2006). Nosocomially acquired *Pseudomonas stutzeri* brain abscess in a child: case report and review. *Infect Control Hosp Epidemiol* 27, 630–632.