Carbapenem-resistant *Pseudomonas aeruginosa* - clonal spread in Southern Brazil and in the State of Goiás

**ABSTRACT**

This study evaluated the clonal spread of carbapenem-resistant *P. aeruginosa* producing SPM-1 type metallo-β-lactamase (MBL), at the university hospital of Florianópolis, Santa Catarina, Brazil, compared to an epidemic clone previously reported, as well as strains collected in other three Brazilian states. Among the isolates, 17 (62%) were clonal and highly related to strains from other regions of Brazil. Six clonal strains harbored the *bla*<sup>SPM-1</sup> gene. The finding of a unique SPM-1 producing clone suggests that its dissemination has contributed to the high resistance to carbapenems in Brazilian hospitals.

**Keywords:** *P. aeruginosa*, carbapenem resistance, metallo-β-lactamase

The isolation of carbapenem-resistant *Pseudomonas aeruginosa* is now common in Brazilian hospitals, and in some institutions this resistance rates reaches close to 50%. In 2003, Gales *et al.*<sup>1</sup> reported the spread of an epidemic clone of SPM-1-type metallo-β-lactamase (MBL) producing *P. aeruginosa* in hospitals located in different Brazilian states. The study analyzed strains from Bahia, Ceará, Distrito Federal, São Paulo and Paraná and found a common PFGE type among carbapenem-resistant *P. aeruginosa* isolates from distinct geographic locations. Clonal dissemination of carbapenem-resistant *P. aeruginosa* was also reported in subsequent studies that included samples from other parts of the country.<sup>2-4</sup>

Clinical isolates of carbapenem-resistant *P. aeruginosa* were first detected in June 2003 at the teaching hospital of the University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil. The increasing frequency of strains with this resistance profile have been isolated prompted us to perform this work, which aiming at evaluating the possible clonal relationship between 29 carbapenem-resistant *P. aeruginosa* isolates from inpatients in this hospital using macrorestriction analysis by pulsed field gel electrophoresis - PFGE [SpeI (10U), CHEF – DRIII (Bio-rad Laboratories, USA)].

The PFGE profiles were compared with those of the SP clone previously described in São Paulo<sup>1</sup> and were also compared with strains collected from other hospitals in Southern Brazil. Some strains from Goiás were also included. Carbapenem-resistance was measured by dilution in agar in accordance with the CLSI (M7-A7) guidelines.<sup>3</sup> Polymerase chain reaction (PCR) for the *bla*<sup>SPM-1</sup> gene was performed according to Sader *et al.*, 2005, and the PCR products were sequenced [GFX-TM PCR purification kit (Amersham Bioscience, NJ, USA), MegaBACE™ (Amersham Bioscience, USA)].

Analysis of the genetic variability of the isolates from Florianópolis over the two-year period from 2003 to 2005 revealed the presence of a clone (clone A) in 62% of the carbapenem-resistant *P. aeruginosa*. The remaining resistant isolates were unrelated to clone A according to Tenover’s criteria.<sup>4</sup> In addition, the present study has also shown that the clone isolated in Florianópolis (SC) is also very closely related to the isolates from Porto Alegre (RS), Curitiba (PR) and Goiânia (GO), as well as to the SP clone described by Gales *et al.* in 2003<sup>1</sup> (Figure 1).
Figure 1: Dendrogram and electrophoretic profiles of PFGE restriction fragments cleaved with the SpeI enzyme from samples of carbapenem-resistant *P. aeruginosa* isolated in Brazil; SP1088: representative sample of the SP clone (São Paulo) (Southeastern region); F1, F4, F5, F7, F11: representative samples of the A clone from Florianópolis/SC (Southern region); POA4, POA43, POA44: representative samples of the clone from Porto Alegre/RS (Southern region); PR5-PR13: isolates from Curitiba/PR (Southern region) (7); GO321, GO321-1, GO516: isolates from Goiânia/GO (Midwestern region).

Of the 17 isolates from Florianópolis characterized as clonal, six (35%) harbored the *bla*<sub>SPM-1</sub> gene. The MIC of these isolates was >128 mg/L for the carbapenems tested (imipenem and meropenem). The 11 remaining clonal isolates were found to have a MIC of 32 mg/L for imipenem, while the MIC for meropenem varied from 64 mg/L to 128 mg/L, indicating the existence of other resistance mechanisms. A study using strains from hospitals in the city of Rio de Janeiro, Brazil, that were characterized as clonal by PFGE, found that SPM-1 was present in 82% of the isolates.

MBL genes have been described as being transferable and therefore capable of disseminating independently among different species of microorganisms. However, we were only able to find SPM-1 in clonal isolates of *P. aeruginosa*, a finding that is similar to those reported in various studies describing the dissemination of SPM in Brazil. 1-3,7,10,13,14,15