Intrahospital spread of carbapenem-resistant *Pseudomonas aeruginosa* in a University Hospital in Florianópolis, Santa Catarina, Brazil

Disseminação Intrahospitalar de *Pseudomonas aeruginosa* em Hospital Universitário de Florianópolis, Santa Catarina, Brasil

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**ABSTRACT**

**Introduction:** Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) has been isolated with increasing frequency in Brazilian hospitals. Since June 2003, its detection in a teaching hospital in the city of Florianópolis, Brazil, has increased. This study aimed to investigate the minimal inhibitory concentration (MIC), presence of Metallo-β-lactamase (MβL) and a possible clonal relationship among the isolates. **Methods:** The study included 29 CRPA and seven isolates with reduced susceptibility. The MIC was determined by agar-dilution. Detection of MβL was performed by Double Disk Synergirom (DDS) and Combined Disk (CD). The MβL gene was verified by PCR and nucleotide sequence analysis. Epidemiological typing was performed by pulse-field gel electrophoresis. **Results:** Among the 29 carbapenem-resistant isolates, polymyxin B presented 100% susceptibility and piperacillin/tazobactam 96.7%. Seventeen (62%) strains were verified as clonal (A clone) and among these, six isolates indicated phenotypically positive tests for MβL and harbored the *bla*\(^\text{IMP-16}\) gene. The first CRPA isolates were unrelated to clone A, harbored *bla*\(^\text{IMP-16}\), and were phenotypically positive only by CD. **Conclusions:** The spread of a high-level of resistance clone suggests cross transmission as an important dissemination mechanism and has contributed to the increased rate of resistance to carbapenems. This study emphasizes the need for continuous surveillance and improved strategies for infection control in this institution.

**Key-words:** *Pseudomonas aeruginosa*. Carbapenem resistance. Nosocomial infections.

**RESUMO**

**Introdução:** O isolamento de *Pseudomonas aeruginosa* resistente aos carbapenênicos (PARC) tem sido cada vez mais frequente nos hospitais brasileiros. O presente estudo investigou a concentração inibitória mínima (CIM), a presença de metallo-β-lactamasas (MβL) e uma possível relação clonal entre PARC isoladas entre junho de 2003 a junho de 2005, em um hospital escola na cidade de Florianópolis, Brasil. **Métodos:** O estudo incluiu 29 PARC e sete isolados com susceptibilidade reduzida. A CIM foi determinada por diluição em ágar. A detecção de MβL foi realizada por sinergismo de duplo disco (SDS) e disco combinado (DC). Genes para MβL foram pesquisados por PCR e confirmados pela análise da sequência de nucleotídeos. A tipagem epidemiológica foi realizada por gel de eletroforese em campo pulsátil. **Resultados:** Entre os 29 isolados resistentes aos carbapenênicos, 100% apresentaram susceptibilidade a polimixina B e 96.7% à piperacilina/tazobactam. Dezessete (62%) destes isolados pertenciam a um mesmo clone (clone A); entre estes, seis isolados apresentaram testes fenotípicos positivos para MβL e carregavam o gene *bla*\(^{\text{IMP-16}}\). O primeiro isolado PARC não foi relacionado ao clone A, carregava o gene *bla*\(^{\text{IMP-16}}\) e foi fenotipicamente positivo somente por DC. **Conclusões:** A propagação de um clone com alto nível de resistência sugere a transmissão cruzada como um importante mecanismo de disseminação e tem contribuído para o aumento nos níveis de resistência aos carbapenênicos. Este estudo enfatiza a necessidade de vigilância contínua e melhoramento das estratégias de controle de infecção nesta instituição.

**Palavras-chave:** *Pseudomonas aeruginosa*. Resistência aos carbapenênicos. Infeccão nosocomial.
showed high-level resistance to carbapenems and seven isolates presented reduced susceptibility. The samples came mainly from patients in the Intensive Care Unit (32.8%) and Internal Medicine Unit III (19.8%), but isolates from other inpatient units were also collected. The bacteria were identified by conventional biochemical tests in accordance with the published recommendations10.

**Antimicrobial susceptibility test**

The MIC of bacterial isolates was determined for each of nine antimicrobial agents (amikacin, cefazidime, aztreonam, cefepime, ciprofloxacin, imipenem, meropenem, piperacillin/tazobactam and polymyxin B), performed by the agar dilution method and interpreted in accordance with CLSI11. *Pseudomonas aeruginosa* ATCC 27853 was used for quality control.

**Phenotypic detection of metallo-β-lactamase**

Two methods were used to screen the isolates for MβL detection: the double-disk synergy test12 (DDS), using 2-mercaptopropionic acid (MPA) (Sigma, Steinheim, Germany), and the ceftazidime disk (CAZ - 30µg), placed 20 mm away; and the combined disk13 (CD) test, using disk of imipenem (IMI - 10µg) with and without ethylenediaminetetraacetic acid (EDTA) (930µg) (Invitrogen, SanDiego, USA). DDS test results were considered positive if the growth inhibition zone increased or if a ghost zone appeared, while the CD test was considered positive if the increase in zone diameter was ≥ 7mm. SPM-1-producing *Pseudomonas aeruginosa* and IMP-producing Acinetobacter baumannii were used as positive controls.

**Molecular detection of metallo-β-lactamase genes**

All isolates were tested for the presence of *bla*<sub>SPM-1</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub> genes by polymerase chain reaction (PCR) using primers, as previously described14. Total DNA was obtained by boiling bacterial cells. PCR conditions used for *bla*<sub>SPM-1</sub> and *bla*<sub>VIM</sub> were performed according to Toleman et al15, while the conditions used to detect the *bla*<sub>IMP</sub> gene were previously described by Gales et al16. Positive controls for *bla*<sub>SPM-1</sub>, *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> genes were run simultaneously.

**Sequencing**

Amplicons obtained from an SPM-producing isolate and from the IMP-producing isolate were sequenced, using the set of primers previously described. The amplification products for the *bla*<sub>IMP</sub> and *bla*<sub>SPM-1</sub> genes were purified using a GFX-TM PCR purification kit (Amersham Bioscience, Piscataway, USA). The sequences were identified with MegaBACE™ (Amersham Bioscience, Piscataway, USA), analyzed with ChromasPro version 1.33 (Techenlysium Pty LTD), and compared with GenBank database sequences using BLAST tool (http://www.ncbi.nlm.gov/BLAST).

**Pulsed-field gel electrophoresis (PFGE)**

DNA of all isolates was prepared as described previously17 and cleaved with SpeI (10U) (Fermentas, Glen Burnie, USA) at 37°C. Electrophoresis was performed on a CHEF - DRIII (Bio-rad Laboratories, Hercules, USA) for 23h at 6V/cm at 12°C and pulse times from 5 to 60 s. The gels were analyzed with Gel-Pro Analyzer 4.0 and NTSYS 2.02 software. Clusters of possibly related isolates were identified using the Dice similarity coefficient and unweighted pair-group method with arithmetic averages (UPGMA). Identical isolates were assigned the same capital letter. Isolates with more than 90% similarity were assigned as a subtype of the major type, which was designated with the same capital letter followed by an Arabic number (e.g. A1, A2, A3, A4).

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**RESULTS**

*Pseudomonas aeruginosa* resistant to carbapenems (imipenem and meropenem), were isolated from the urinary tract (37.9%), bloodstream (31%), respiratory tract (13.8%) and from other anatomical sites (17.3%). All of them were susceptible to polymyxin B, and 96.7% to piperacillin/tazobactam. Susceptibility to the other antimicrobial agents tested was infrequent, 6% to aztreonam and cefepime; 10% to ciprofloxacin; 16.7% to amikacin and 43% to ceftazidime. Six out of the seven isolates that showed reduced susceptibility to carbapenems presented reduced susceptibility only to meropenem (MIC 8µg/mL), while the remaining isolate showed reduced susceptibility to both carbapenems tested (meropenem and imipenem) (MIC 8µg/mL).

Isolates that showed intermediate susceptibility to carbapenems presented negative results in the phenotypic test for MβL. When the 29 carbapenem-resistant isolates were tested, seven MβL-producing isolates were detected by the CD test and six isolates by DDS. When PCR was used to detect MβL genes, six of the seven isolates phenotypically positive for MβL yielded a 650 bp product compatible with a fragment amplified from *bla*<sub>SPM-1</sub>, while one isolate yielded a 590 bp product compatible with a fragment amplified from *bla*<sub>IMP</sub>. The remaining isolates did not generate PCR products. The results of PCR for *bla*<sub>SPM-1</sub> confirmed the findings of both phenotypic methods.


**DISCUSSION**

*Pseudomonas aeruginosa* was one of the main pathogens involved in nosocomial infection during the study period. Carbapenems were considered an excellent therapeutic choice for treatment of these infections; however, the increasing resistance to these agents verified in the institution (13% in 2003, 32% in 2004 and 44% in 2005) was concerning.

All isolates were susceptible to polymyxin B, currently used for empirical treatment of *Pseudomonas aeruginosa* infections in severely ill patients, particularly those in Intensive Care Units in Brazilian hospitals. Piperacillin-tazobactam was also a viable alternative treatment (96.7% susceptibility) and all isolates producing MβL were susceptible to this drug. According to previous studies, piperacillin-tazobactam could be a reliable treatment option for MβL-producing *Pseudomonas aeruginosa* when used appropriately. Expression of high-level resistance to various antimicrobial agents may involve

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**TABLE 1 - Genotypic and phenotypic data for the characterisation of 29 isolates of carbapenem-resistant *Pseudomonas aeruginosa* used in this study.**

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different mechanisms, such as the production of enzymes, a reduction in the permeability of the external membrane and overexpression of efflux systems.\textsuperscript{2,20,21}

MβL-producing isolates presented high MICs for both carbapenems (>-128 µg/mL) and other β-lactams (≥256 µg/mL), except aztreonam (16 µg/mL). Aztreonam is not a good substrate for MβLs, including SPM-1\textsuperscript{21}, however, its reduced susceptibility could be explained by the possible additional mechanisms of resistance to β-lactams carried by this strain.\textsuperscript{4} The isolates belonging to clone A, which do not carry MβLs, showed lower MICs, especially to Imipenem (32 to 64 µg/mL), and somewhat higher for meropenem (64 to 128 µg/mL). The overexpression of the MexAB-OprM efflux system could explain this finding, since the hydrophobic chains of meropenem seem to be a better substrate than imipenem in this system.\textsuperscript{22} Four isolates sensitive to ceftazidime (MIC 8 µg/mL), were resistant to cepime and carbapenems. According to Hocquet et al,\textsuperscript{23} this phenotype could be due to overexpression of ampC and the MexXY-OprM\textsuperscript{24} efflux system. This clone probably has multiple resistance mechanisms, which, according to Maniati et al,\textsuperscript{25} would explain the high MIC for carbapenems.\textsuperscript{21}

Unrelated isolates presented variable susceptibility profiles, some of which showed resistance only to carbapenems. A possible resistance mechanism of these isolates could be due to the loss of porin (OprD).\textsuperscript{26} The expression of different resistance mechanisms to \textit{Pseudomonas aeruginosa} reveals the diverse sensitivity of phenotypic profiles in the susceptibility test of this microorganism.

Attempts to standardize phenotyping techniques to detect MβL have encountered various obstacles, such as the differences observed among this class of enzymes and the variation in test results according to the species of bacteria studied. Picão et al\textsuperscript{27} suggested that DDS is the best method for testing different species of bacteria with diverse MβLs.\textsuperscript{28} The phenotypic methods used in this study proved to be satisfactory in identifying SPM-1-producing isolates, the main MβL detected in Brazilian hospitals. Nevertheless, DDS failed to identify the isolate carrying bla\textsubscript{SPM-1-16}. The IMP-16 enzyme was characterized in a \textit{Pseudomonas aeruginosa} isolate in a hospital in the City of Brasilia in 2001, \textit{Pseudomonas aeruginosa} which produces this enzyme was also isolated in Santa Catarina in 2003, suggesting that the bla\textsubscript{SMP-1-16} variant is circulating in Brazil. These findings justify the use of both methods, DDS and CD, to improve the sensitivity of MβL phenotyping.

One clonal type (clone A) was verified as predominant among the carbapenem-resistant isolates and was identified in all units at the HU/UFSC. This finding suggests that this clone is better adapted to the hospital environment. Alternatively, a continuous source of bacteria with diverse MβLs may be circulating in Brazil. These findings justify the use of both methods, DDS and CD, to improve the sensitivity of MβL phenotyping.

The authors thank Laboratório Alerta, Division of Infectious Diseases, São Paulo School of Medicine (EPM/UNIFESP) Federal University of São Paulo, for providing the control strains.
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