Short Communication

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Production of metallo-β-lactamase among *Pseudomonas aeruginosa* strains isolated in the State of Sergipe, Brazil

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ABSTRACT

Introduction: Acquired production of metallo-β-lactamases is an important mechanism of resistance in *Pseudomonas aeruginosa*. The objective of this study was to investigate the production of metallo-β-lactamase and the genetic diversity among ceftazidime-resistant *P. aeruginosa* isolates from State of Sergipe, Brazil. **Methods:** Metallo-β-lactamase was investigated using the disk approximation test and polymerase chain reaction (PCR). Genetic diversity was evaluated by pulsed-field gel electrophoresis (PFGE). **Results:** A total of 48 (51.6%) isolates were resistant to ceftazidime. Six (12.2%) of these were positive for metallo-β-lactamase production. Only two (4.1%) of the ceftazidime-resistant isolates carried the bla_{SPM-1} gene. **Conclusions:** Production of metallo-β-lactamases was not the main mechanism of resistance to ceftazidime and carbapenems among *P. aeruginosa* strains in Sergipe, Brazil.

Keywords: *Pseudomonas aeruginosa.* Antimicrobial resistance. Metallo-β-lactamase.

Carbapenem resistance has increased among *Pseudomonas aeruginosa* strains worldwide. Production of metallo-β-lactamases (MβL) has been identified as an important mechanism of carbapenem resistance among *P. aeruginosa*(1).

Seven types of acquired MβL, designated as IMP (imipenemase), VIM (Verona integron-encoded metallo-β-lactamase), SPM (São Paulo metallo-β-lactamase), GIM (German imipenemase), AIM (Adelaide imipenemase), NDM (New Delhi metallo-β-lactamase)(1), and FIM (Florence imipenemase)(2), have been identified in *P. aeruginosa*. In Brazil, the predominant MβL-encoding gene is bla_{SPM-1}; this gene was originally described in an isolate from São Paulo and was later detected among *P. aeruginosa* isolates from numerous cities in Brazil. These SPM-1-positive isolates are predominantly related to a single clone designated the São Paulo (SP) clone(3). Although some studies have reported the occurrence of *P. aeruginosa*-producing MβL in Northeastern Brazil(4) (5), studies concerning the occurrence and spread of MβL-producing isolates in this region of Brazil remain scarce.

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Thus, the objective of the present study was to investigate the occurrence of MβL-producing strains and the genetic diversity of ceftazidime-resistant *P. aeruginosa* isolates from three institutions in the State of Sergipe, Brazil.

Pseudomonas aeruginosa isolates (n = 95) were recovered from March 2008 to December 2009 from patients attending two tertiary healthcare institutions [*Hospital Primavera* (HP), a private hospital with 45 (47.4%) isolates and Hospital de Urgência do Estado de Sergipe (HUSE), a public hospital with 10 (10.5%) isolates], and from the *Laboratório Central de Saúde Pública do Estado de Sergipe* (LACEN), a public clinical laboratory, which receives clinical specimens from patients attending hospitals throughout Sergipe with 40 (42.1%) isolates. Most (62.1%) isolates were from patients admitted to the intensive care units of the two hospitals included in this study. Isolates were obtained from the following clinical specimens: 24 (25.3%) from lower respiratory tract secretions, 21 (22.1%) from urine, 11 (11.6%) from cerebrospinal fluid, eight (8.4%) from surgical wound secretions, three (3.2%) from blood, and 28 (29.4%) from various other clinical sources. Only one isolate was selected from each patient.

Pseudomonas aeruginosa isolates were identified using a VITEK 2 automated system (bioMerieux S. A., France). Antimicrobial susceptibility was determined by disk diffusion, according to the Clinical Laboratory Standard Institute (CLSI) recommendations(6). The following antimicrobials were tested: amikacin, aztreonam, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, and piperacillin/tazobactam

(CECON, São Paulo, Brazil). *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218 were used as quality controls.

Isolates with resistance to ceftazidime (CAZ-R) were phenotypically screened for MβL production using the disk approximation (DA) test, as described by Arakawa et al.[7]. Undiluted 2-mercaptopropionic acid (2-MPA) was used as an inhibitor of MβL, and a 30-μg ceftazidime disk was used as the substrate. For the DA test, 3μL of 2-MPA was added to a blank filter disk, placed 2cm away from a ceftazidime disk. One ceftazidime disk was also placed 5 cm away. After incubation, the presence of an enlarged zone of inhibition was interpreted as a positive test. A metallo-β-lactamase-producing strain (P1088) and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively, for the MβL phenotypic screening tests.

CAZ-R isolates were also subjected to conventional polymerase chain reaction (PCR) using specific primers to detect the carbapenemase-encoding genes bla_{SPM-1}[8], bla_{IMP-1}[9], bla_{VIM-2}[10], and bla_{GIM-1}[9]. Positive controls carrying each of the genes investigated were included in the PCR detection.

Pseudomonas aeruginosa CAZ-R isolates were typed by pulsed-field gel electrophoresis (PFGE) using SpeI (Invitrogen, São Paulo, Brazil), as described elsewhere[11]. Band profiles were analyzed using the Gel-Compar II Program (Applied Maths, Kortrijk, Belgium). Isolates with a coefficient of similarity of 85% of more (with PFGE patterns differing in one to six bands) were included in the same genotype designated with a capital letter. A bla_{SPM-1}-positive *P. aeruginosa* strain representative of the SP clone (P1088) was included for comparison. A clinical bla_{SPM-1}-positive strain isolated in Niterói, State of Rio de Janeiro (365h2) that was part of our culture collection was also used for comparison.

A total of 49 (51.6%), 22 (23.2%), and 19 (20%) isolates were resistant to ceftazidime, imipenem, and meropenem, respectively. The resistance rates for the other antibiotics tested were as follows: amikacin, 16.8%; gentamicin, 20%; ciprofloxacin, 27.4%; piperacillin/tazobactam, 32%; cefepime, 35.8%; and aztreonam, 45.3%.

Phenotypic tests were positive in six (12.2%) of the 49 CAZ-R isolates using the CAZ-2MPA combination. However, based on the genes screened by PCR, only two (4.1%) isolates carried the bla_{SPM-1} gene. Interestingly, one of these bla_{SPM-1}-positive isolates [isolate 65, carbapenem-susceptible, with a minimum inhibitory concentration (MIC) for imipenem of 0.75μg/mL, as determined by E-test] yielded a positive DA test result, while the other (isolate 69, carbapenem-resistant) was negative. The phenotypic tests of both SPM-1-positive isolates were repeated. No other carbapenemase-encoding genes were found. The two SPM-1-positive isolates showed resistance to six or seven antimicrobial agents. The characteristics of the six Mβl-positive isolates according to the phenotypic tests are shown in **Table 1**.

A total of 41 of the 49 CAZ-R isolates were analyzed by PFGE. Thirty-seven unique PFGE band profiles were identified (**Figure 1**); among these, genotypes A, D, and H included three isolates each, while genotypes B, C, E, F, G, and I included two isolates each. The three isolates belonging to genotype A were isolated from the three institutions involved in the study between August and September 2009. The two bla_{SPM-1}-positive isolates (65 and 69) included in genotype F were obtained from two different sectors of HP and were genotypically related to the bla_{SPM-1}-positive controls strains (> 95% similarity). One band differed among isolates 65 and 69. The bla_{SPM-1}-positive control P1088 and isolate 65 exhibited indistinguishable PFGE patterns, whereas isolate 69 and the other bla_{SPM-1}-positive control strain (365h2) were also indistinguishable by PFGE (**Figure 2**).

Detection of MβL-producing *P. aeruginosa* is important for controlling the dissemination of MβL isolates and for the correct choice of antimicrobial regimens; these enzymes are able to hydrolyze most β-lactams but do not always exhibit carbapenem resistance[12], as observed in one of the two SPM-1-positive isolates. This result emphasizes the utilization of ceftazidime resistance as a criterion for selecting isolates for phenotypic MβL production tests. Among the CAZ-R isolates, six (12.2%) were positive for MβL production according the phenotypic tests results. However, only two isolates carried an MβL-encoding gene, one of which was considered negative in the DA tests, suggesting that these tests were not useful for screening MβL in the isolates studied, yielding false-positive and false-negative results. This discrepancy among the results

TABLE 1 - Characteristics of six positive metallo-β-lactamases strains according to phenotypic tests.

Strains	Antimicrobial resistance	DA-test CAZ/2-MPA	bla_{SPM-1} PCR	Genotype PFGE
6	CAZ, CEF, AMI, GEN	Positive	Negative	NT
7	CAZ, CEF	Positive	Negative	Unique pattern
12	CAZ, CEF, GEN	Positive	Negative	C
16	CAZ, CEF, ATZ, PIP/TAZ	Positive	Negative	E
55	CAZ, CEF, PIP/TAZ, CIP, IMP, MER, GEN	Positive	Negative	Unique pattern
65	CAZ, CEF, PIP/TAZ, CIP, GEN	Positive	Positive	F

DA: disk approximation; CAZ: ceftazidime; MPA-2: 2-mercaptopropionic acid; PCR: polymerase chain reaction; PFGE: pulsed-field gel agarose; CEF: cefepime; AMI: amikacin; GEN: gentamicin; AZT: aztreonam; PIP/TAZ: piperacillin/tazobactam; CIP: ciprofloxacin; IMP: imipenem; MER: meropenem; NT: nontyped (DNA degradation).

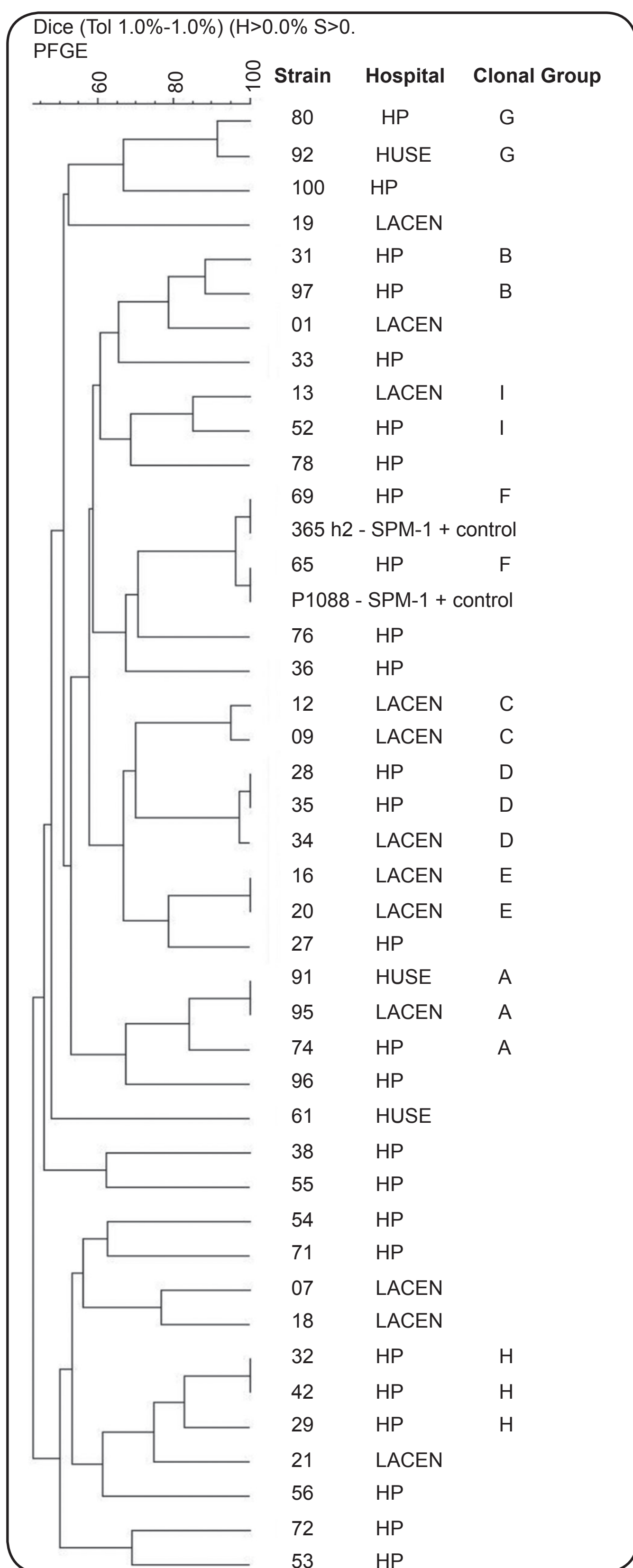

FIGURE 1 - Dendrogram from computer analysis of pulsed-field gel electrophoresis profiles of 41 ceftazidime-resistant *Pseudomonas aeruginosa* clinical isolates from State of Sergipe and two SPM-1-positive control strains (P1088 and 365h2). PFGE: pulsed-field gel agarose; HP*: Hospital Primavera;* HUSE: *Hospital de Urgência do Estado de Sergipe;* LACEN: *Laboratório Central de Saúde Pública do Estado de Sergipe;* SPM: São Paulo metallo-β-lactamases.

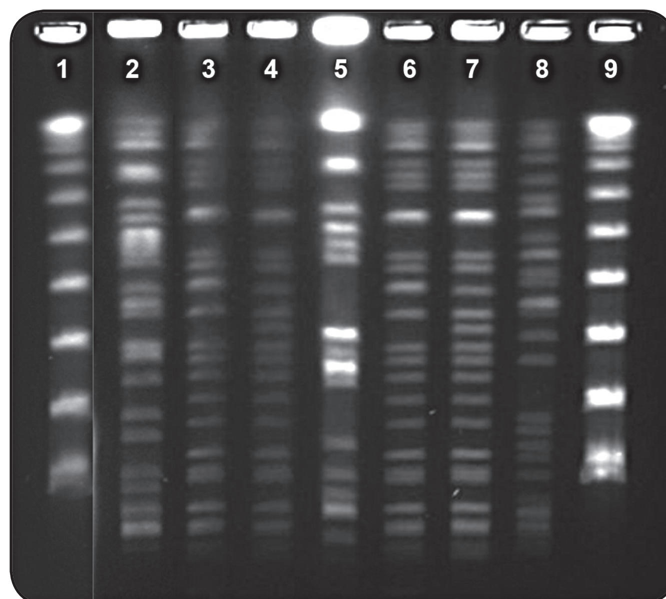

FIGURE 2 - Representative gel electrophoretic profiles of pulsed-field gel agarose analysis of ceftazidime-resistant *Pseudomonas aeruginosa* isolates obtained after digestion with *Spe*I. Lanes 1 and 9: molecular weight marker; lanes 3 and 4: isolates 69 and 65 (clonal group F bla_{SPM-1} positive); lanes 6 and 7: P1088 and 365h2 (bla_{SPM-1}-positive control strains); lanes 2, 5, and 8: unique patterns**.**

obtained in phenotypic and genotypic tests has also been reported in other studies[13] [14] and may be related to difficulties in reading and interpreting tests, variations in the sensitivity and specificity of the method according to the combination of substrate and chelating agent, the concentration of the chelating agent, and the presence of other resistance mechanisms that may affect the results of the phenotypic tests.

We found a low (4.1%) rate of isolates harboring the bla_{SPM-1} gene. Different rates of SPM-producing *P. aeruginosa* isolates have been reported in Brazil, varying according to geographic region as follows: 7.5% in São Luis[5] (in Northeastern Brazil), 20.7% in Recife[4] (in Northeastern Brazil), and 42% in Goiás[13] (in Mid-west Brazil).

The two SPM-1-positive isolates showed electrophoretic profiles indistinguishable from or closely related to the bla_{SPM-1}-positive control strains (P1088 and 365h2). These results confirmed the data from other studies indicating that the SP clone was widespread in Brazil[3]. However, the MβL-negative isolates showed high genetic diversity and were unrelated to the SP clone. Interestingly, one of the bla_{SPM-1}-positive isolates was susceptible to carbapenem. One possible reason for this could be the low expression of bla_{SPM-1}. A carbapenem-susceptible *P. aeruginosa* isolate harboring the bla_{SPM-1} gene has previously been described in Brazil[15].

To the best of our knowledge, this is the first report of SPM-1 *P. aeruginosa* isolates identified in Sergipe. However, the results obtained in the present study suggested that resistance to ceftazidime and carbapenem among the *P. aeruginosa* isolates analyzed in this study was a result of resistance mechanisms other than MβL production.

Ethical considerations

This study was approved by the Research Ethics Committee of the School of Medicine of *Universidade Federal Fluminense* (UFF).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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