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Major Article

Clinical and microbiological features of infections caused by *Pseudomonas aeruginosa* in patients hospitalized in intensive care units

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Abstract

Introduction: The spread of multidrug-resistant *Pseudomonas aeruginosa* in Brazilian hospitals has greatly impacted upon the morbidity and mortality of individuals in intensive care units. Given the lack of information regarding the dynamics of multidrug resistance in northern Brazil, we analyzed the clinical and microbiological features of nosocomial infections caused by *P. aeruginosa*. **Methods**: Between January 2010 and March 2012, we conducted a retrospective cohort study of *P. aeruginosa* isolates from 54 patients who were hospitalized in intensive care units. The clinical and epidemiologic variables were analyzed, including the patients' demographic data and comorbidities, and the lengths of the intensive care unit stays, the classification of the infections as nosocomial, the use of invasive procedures, antimicrobial therapy, and the patients' outcomes. We undertook susceptibility tests, molecular detection of the metallo-β-lactamase genes, and genotypic analyses of the isolates using the repetitive element-polymerase chain reaction. **Results:** Multidrug resistance occurred most frequently among isolates from adults who had been hospitalized for an average of 87.1 days. The use of mechanical ventilation and urinary catheters were risk factors for infection. The four isolates that harbored the *bla*SPM-1-like gene showed >95% genetic similarity. **Conclusions**: This study's findings show that *P. aeruginosa* has a high death rate, and that inadequate treatment and invasive procedures are risk factors for infection. This is the first report describing the detection of the *bla*SPM-1-like gene in northern Brazil. These results highlight the need for better monitoring and a greater understanding of nosocomial infections and their public health impacts.

Keywords: *Pseudomonas aeruginosa*. Nosocomial infection. Antimicrobial resistance. Clinical features. Intensive care unit.

INTRODUCTION

The frequency of infections caused by *Pseudomonas aeruginosa* has increased in conjunction with increases in their morbidity and mortality among hospitalized patients, all of which are exacerbated by antimicrobial resistance[1] [2] [3]. The findings from multicentric studies have demonstrated that resistance to carbapenems, aminoglycosides, and fluoroquinolones has increased gradually over the past few years[2] [4] [5] [6] [7].

The impact of *P. aeruginosa* resistance on health systems is a major concern in hospitals in Brazil and worldwide, and it is mainly caused by strains that produce metallo-β-lactamases (MBLs). This resistance mechanism has spread across hospitals because of the frequent use of carbapenems, which were considered the only effective antibiotic against *P. aeruginosa* infections[5] [8]. In Brazil, several MBL-producing multidrug-resistant strains have been isolated, and *bla*SPM-1, which was initially detected in São Paulo, is the most prevalent gene[6] [9] [10].

The widespread dissemination of MBL-producing multidrug-resistant strains across Brazilian hospitals highlights the need for national data, particularly from northern Brazil where investigations into the patients' clinical characteristics and the microbiological features of the infections remain scarce. Hence, we aimed to analyze the clinical, epidemiologic, and molecular characteristics of *P. aeruginosa* infections in patients who were hospitalized in intensive care units (ICUs) in a teaching hospital in Belém, Pará, Brazil.

METHODS

Study design and patients

A retrospective cohort study was conducted in which individuals were categorized as exposed if they had been infected

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by multidrug-resistant *Pseudomonas aeruginosa* (MDRPA), or not exposed if they had been infected by non multidrug-resistant *P. aeruginosa* (non-MDRPA). The study population comprised patients who had been hospitalized in adult, pediatric, and neonatal ICUs within a teaching hospital in Belém, Pará, Brazil. Given the more comprehensive analysis of the data, the pediatric and neonatal ICUs were merged to form one group called NeoPed.

The clinical and epidemiologic variables analyzed were the demographic data, namely, gender and age, the comorbidities, the length of the ICU stay, classification as a hospital-acquired infection (HAI) using the epidemiologic criteria established by the Centers for Disease Control and Prevention, the use of invasive procedures, adequate or inadequate antimicrobial therapy, the outcome, namely, discharge or death, the McCabe Jackson Score, which verifies the relationship between disease severity at admission and the occurrence of a HAI, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and the Charlson Comorbidity Index (CCI) score.

Bacterial isolates and identification

The samples were collected from January 2010 until March 2012, and they were recovered from blood, urine, bronchoalveolar washes, tracheal secretions, and central vascular catheters. All of the *P. aeruginosa* isolates were plated onto MacConkey Agar and incubated for 24h at 35°C. Bacterial identification was performed using a Vitek® 2 microbial identification system (Vitek® 2 software, version R02.03, Advanced Expert System software, version R02.00N; bioMérieux, Marcy l'Etoile, France), which was complemented by biochemical and phenotypical tests, including the oxidase test, the absence of glucose fermentation on triple sugar iron agar, pigment production, the oxidative behavior on Hugh Leifson medium, and the cytochrome oxidase reaction.

Antimicrobial susceptibility testing

The broth microdilution method used was based on the Clinical and Laboratory Standards Institute's (CLSI) reference method[11]. To ensure quality control, the following samples from the American Type Culture Collection (ATCC) were used: *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The antibiotics tested were amikacin (AM), gentamicin (GN), piperacillin-tazobactam (PTZ), cefepime (CPM), ciprofloxacin (CIP), imipenem (IPM), meropenem (MEM), ceftazidime (CAZ), and polymyxin B (POL).

Molecular detection of the *bla*-variant genes and molecular typing

The molecular detection of the *bla*SPM-1, *bla*IMP-1, and *bla*VIM-1 MBL genes was performed using conventional or multiplex polymerase chain reactions (PCRs). **Table 1** presents the oligonucleotide and amplicon sequences used in the PCRs[10] [12] [13] [14]. The genotypic analysis of the isolates was performed using repetitive element (rep)-PCRs and the DiversiLab® System (bioMérieux). Strains with similarities of ≥97% were considered to have unique patterns.

Statistical analyses

The categorical variables were compared using the chi-squared test, Fisher's test, and the Mantel-Haenszel test, and bivariate analyses and multivariate analyses with logistic regression were used to evaluate the effects of the individual potential risk factors on infection by MDRPA or non-MDRPA. The odds ratios (ORs) and the 95% confidence intervals (CIs) were estimated, and a p-value <0.05 was considered statistically significant. We have used BioEstat 5.0.

Ethical considerations

The study was approved by the ethics committee at Fundação Santa Casa de Misericórdia do Pará (0086.0.440.000-10, number 072 Protocol/2010).

RESULTS

The hospital that was involved in this study is a 395-bed tertiary care teaching hospital. Of the 54 patients with

TABLE 1 - The oligonucleotide primers used for metallo-β-lactamase-encoding gene amplification.

Gene	Sequence (5'–3')	Amplicon size (bp)	Reference
*bla*IMP	F: CTACCGCAGCAGAGTCTTTGC		
	R: GAACAACCAGTTTTGCCTTACC	587	12
*bla*VIM	F: TCTACATGACCGCGTCTGTC		
	R: TGTGCTTTGACAACGTTCGC	920	13
*bla*VIM	F: ATGTTCAAACTTTTGAGTAGTAAG		
	R: CTACTCAACGACTGAGCG	801	13
*bla*SPM	F: CCTACAATCTAACGGCGACC		
	R: TCGCCGTGTCCAGGTATAAC	650	10
*bla*SPM	F: CCTACAATCTAACGGCGACC		
	R: TCGCCGTGTCCAGGTATAAC	650	14

bp: base pair; **F**: forward. **R:** reverse.

TABLE 2 - Bivariate analysis of the clinical features of the patients with multidrug-resistant *Pseudomonas aeruginosa* infections between January 2010 and March 2012.

Variable	MDRPA		Non-MDRPA		Odds ratio (95% CI)	Chi-square test	p value
	n	%	n	%			
Hospital ICU							
adult	16	53.3	14	41.2	5.7143 (1.5712–20.7820)		**0.0128**
neoped	4	16.7	20	83.3	0.1750 (0.0481–0.6364)		**0.0128**
Age range (years)							
0–1	3	14.3	18	85.7	0.1569 (0.0387–0.6381)		**0.0134**
1–10	0	0	1	2.9		0.0734	0.7864
11–20	2	10.0	2	5.9	1.7778 (0.2304–13.7174)		0.9841
21–60	6	30.0	7	20.5	1.6531 (0.4655–5.8697)		0.6515
>60	9	45.0	6	17.6	3.8152 (0.0979–13.2791)		0.0639
Length of ICU stay							
≤30 days	2	10.0	9	26.5	0.3086 (0.0594–1.6033)		0.2707
≥30 days	18	90.0	25	73.5	0.6237 (0.6237–6.8309)		0.2707
McCabe Jackson score							
potentially fatal underlying disease	13	65.0	11	32.3	3.8831 (1.2095–12.4668)		**0.0405**
nonfatal underlying disease	3	15.0	3	8.8	1.8235 (0.3311–10.0435)		0.4855
Invasive procedures							
central vascular catheter	20	39.2	31	60.8		0.5052	0.4521
mechanical ventilation	20	100.0	25	73.5		4.5900	**0.0321**
urinary catheter	12	60.0	10	29.4	3.6000 (1.1291–11.4786)		**0.0273**
hemodialysis	4	20.0	1	2.9	8.2500 (0.8513–79.9538)		0.1090
gastrectomy	2	10.0	1	2.9	3.6667 (0.3107–43.2730)		0.6323
Sites of HAIs							
pulmonary	19	95.0	22	70.5	10.3636 (1.2311–87.2422)		**0.0288**
bloodstream	18	90.0	30	88.24	1.2000 (0.1993–7.2247)		0.8033
urinary tract	13	65.0	8	25.8	6.0357 (1.7938–20.3090)		**0.0063**
skin and soft tissues	0	0	3	9.1		0.5652	0.4521
Deaths							
all units	14	70.0	20	58.9	1.6333 (0.5043–5.2901)		0.5964
adult ICU	13	81.3	10	71.4	1.73 (0.31–9.57)		0.5327
NeoPed ICU	1	25.0	11	55.0	0.27 (0.02–3.09)		0.2835
inadequate therapy	20	100.0	19	55.8		10.1171	**0.0014**
Antibiotic use							
amikacin	4	25.0	22	57.8	0.1364 (0.0371–0.5014)		**0.0038**
gentamicin	1	6.7	14	93.3	0.0752 (0.0090–0.6287)		**0.0107**
cefepime	13	40.6	19	59.3	1.4662 (0.4683–4.5904)		0.7100
ciprofloxacin	5	26.3	14	73.7	0.4762 (0.1404–1.6146)		0.3644
imipenem	17	51.5	16	48.4	6.3750 (1.5720–25.8522)		**0.0134**
meropenem	4	20.0	16	80.0	0.2813 (0.0777–1.0177)		0.0897
piperacillin-tazobactam	10	33.3	20	66.7	0.7000 (0.2304–2.1266)		0.7289

MDRPA: multidrug-resistant *Pseudomonas aeruginosa*; **non-MDRPA**: non-multidrug-resistant *Pseudomonas aeruginosa*. **CI**: confidence interval; **ICU**: intensive care unit; **HAIs**: hospital-acquired infections.

P. aeruginosa infections, comprising 51.9% (28/54) female and 48.1% (26/54) male patients, 55.6% (30/54) were hospitalized in the adult ICU and 44.4% (24/54) were hospitalized in the NeoPed ICU. The bacterial isolates were recovered from urinary tract infections (UTIs) in 9.3% (5/54), respiratory tract infections (RTI) in 44.4% (24/54), and bloodstream infections (BSI) in 46.3% (25/54) of the patients.

The frequency of the MDRPA strains was 37% (20/54) and the frequency of the non-MDRPA strains was 63% (34/54). Regarding the detection of the genes associated with MBL production, 20% (4/20) of the isolates were positive for the *bla*SPM-1-like gene, and the *bla*IMP-like and *bla*VIM-like genes were not detected.

A higher incidence of MDRPA strains was evident in the patients who were aged >60 years and were in the adult ICU (OR: 5.7143; p-value = 0.0128) (**Table 2**). The average length of stay in the ICUs was 87.1 days and the median length of stay in the ICUs was 62 days, specifically, 67.8 days in the adult ICU and 111.3 days in the NeoPed ICU. Despite a high rate of death as a consequence of the *P. aeruginosa* infections in the hospital ICUs, with mortality rates of 70% for patients with MDRPA and 70.5% for patients with non-MDRPA, the bivariate analysis did not demonstrate an association between death and MDRPA infection when the ICUs were analyzed together (p-value = 0.5964) or separately (adult ICU: p-value = 0.53272; NeoPed ICU: p-value = 0.28354).

Mechanical ventilation (chi-squared test: 4.5900; p-value = 0.0321) and urinary catheters (OR: 3.6000; p-value = 0.0273) were associated with *P. aeruginosa* infections. All of the infections were confirmed as HAIs and they were associated with RTIs (OR: 10.3636; p-value = 0.0288) and UTIs (OR 6.0357, p-value = 0.0063) (**Table 2**).

Regarding empirical therapy, inadequate therapy applied at the time of diagnostic suspicion (chi-squared test: 10.1171; p-value = 0.0014) and the administration of AM (OR: 0.1364; p-value = 0.0038) or GN (OR: 0.0752, p-value = 0.0107) were associated with a lower risk of MDRPA infection, and the administration of IMP showed a significant association with

TABLE 3 - Bivariate analysis of the severity scores of the patients with *Pseudomonas aeruginosa* infections in the adult intensive care unit.

	MDRPA n	Non-MDRPA n	Odds ratio (95% CI)	p value
APACHE II score			0.9259 (0.2608–3.2868)	0.8395
Average 21 points				
≤20 points*	5	9		
>20 points*	11	5	7.0889 (1.9417–25.8800)	0.0047
Score				
0–2 points	15	13	4.8462 (1.4223–16.5122)	0.0198
3–6 points	1	1	0.7368 (0.1026–29.3947)	0.7194

CI: confidence interval; **MDRPA**: multidrug-resistant *Pseudomonas aeruginosa*; **non-MDRPA**: non-multidrug-resistant *Pseudomona aeruginosa*. **APACHE**: Acute Physiology Chronic Health Evaluation. *Charlson Comorbidity Index.

TABLE 4 - Susceptibility profiles of 54 *Pseudomonas aeruginosa* isolates recovered from the intensive care units.

	Sensitive		Resistant		Total		
Antibiotic	**n**	**%**	**n**	**%**	**n**	**MIC 50%**	**MIC 90%**
Cefepime	28	51.9	26	48.1	54	16	128
Imipenem	19	35.2	35	64.8	54	8	16
Meropenem	19	35.2	35	64.8	54	2	8
Piperacillin-tazobactam	29	53.7	25	46.3	54	8	32
Gentamicin	29	53.7	25	46.3	54	4	64
Ciprofloxacin	32	59.3	22	40.7	54	4	64
Amikacin	46	85.2	8	14.8	54	4	32
Ceftazidime	20	37.0	34	63.0	54	32	64
Polymyxin B	54	100.0	0	0	54	0.5	1

MIC: minimum inhibitory concentration; **n**: number of samples.

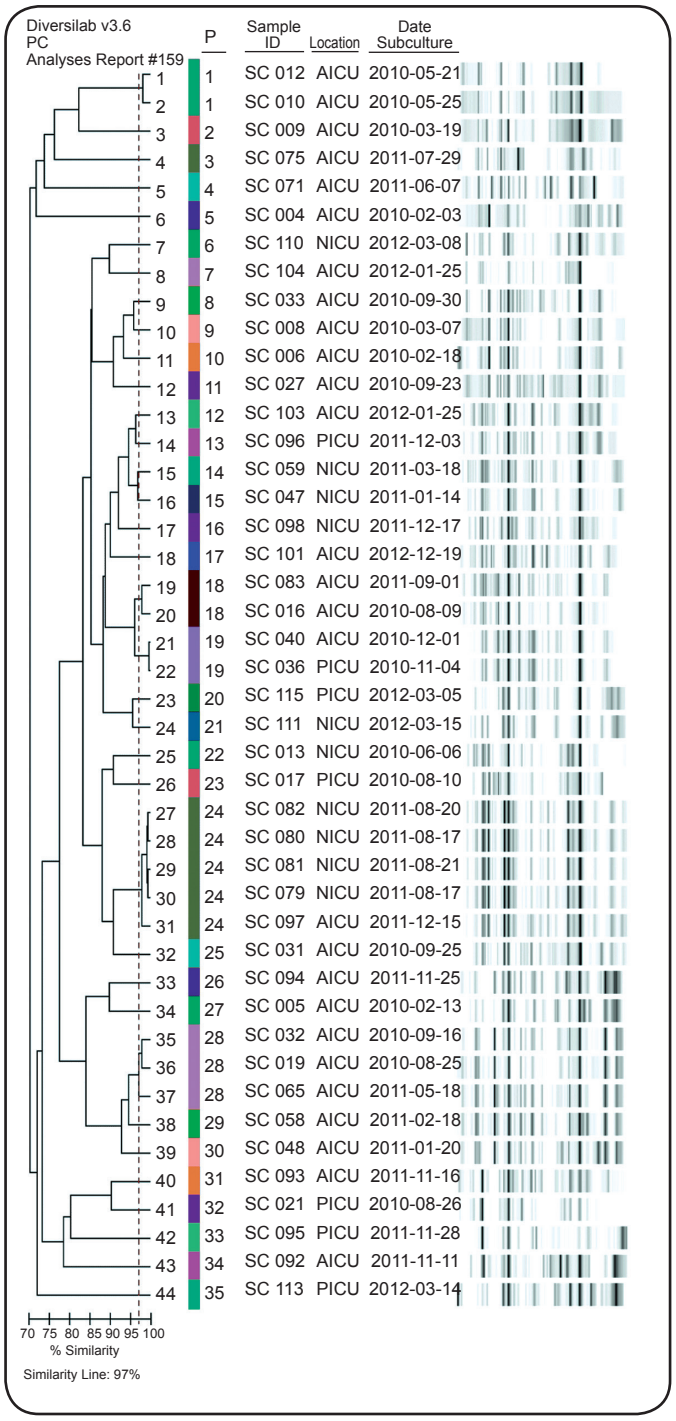

FIGURE 1 - Dendrogram of the clonal relations among the *Pseudomonas aeruginosa* strains. P: pattern ≥ 97% similarity; **Sample ID**: identification of the isolate; **ICU**: intensive care unit; **Subculture date**: date of culture; **AICU**: adult ICU; **PICU**: pediatric ICU; **NICU**: neonatal ICU.

MDRPA (OR: 6.3750; p-value = 0.0134) (**Table 2**). There were no statistically significant associations between MDRPA infection and the comorbidities or the number of antimicrobials used per class. Bivariate analyses determined relationships between MDRPA infection and APACHE II scores of >20 points and CCI scores of between 0 and 2 points (**Table 3**).

The multivariate analysis demonstrated an association between sepsis and death (OR: 16.3252; p-value = 0.0122). Mechanical ventilation was not significantly associated with death and IPM exposure was significantly associated with death (OR: 4.9378; p-value 0.0333). **Table 4** presents the susceptibility profiles, including the percentage values and the minimal inhibitory concentration (MIC) 50% and MIC 90% values, for each drug. All of the isolates were sensitive to POL.

Four of the *P. aeruginosa* isolates harbored the *bla*SPM-1-like gene. The *bla*IMP-1-like and *bla*VIM-1-like gene variants were not detected. All of the isolates that were positive for the *bla*SPM-1-like gene were phenotypically resistant to AM, GN, PTZ, CPM, CIP, IPM, MEM, and CAZ.

Forty-four isolates were genotyped using rep-PCR, and 35 patterns (P) were obtained with a similarity index of ≥97% (**Figure 1**). The data suggest persistent clones in the same area, in different patients (P1, P18, P19, and P24) and in different ICUs (P19 and P24). Isolates that were positive for the *bla*SPM-1-like gene (P18, 19, and 20) were detected that originated from the pediatric ICU and two shared single standard isolates (P19) were detected that originated from the adult ICU. (**Figure 1**). P16 was shared by two strains, namely, SC083 and SC016, which, despite being >97% similar, differed because of the presence of the *bla*SPM-1-like gene (**Figure 1**). The P24 and P28 clones were observed over several months in the ICUs. Nine clonal strains (SC12, SC10, SC80, SC81, SC79, SC97, SC32, SC19, and SC65) that shared P1, P24, and P28 were associated with death.

DISCUSSION

The data from this study are the first from Northern Brazil that describe the clinical, epidemiologic, and molecular characteristics of infections caused by MBL-producing *P. aeruginosa* that was harboring the *bla*SPM-1-like gene, a variant that is widespread among Brazilian hospitals.

An analysis of the distribution of the HAIs and the infection sites determined a higher prevalence of lower RTIs that were associated with pulmonary diseases within the adult population, which highlights mechanical ventilation as a risk factor. In ICUs, most nosocomial pneumonia is caused by Gram-negative non-fermenting bacilli, including *P. aeruginosa*, and, as a consequence of mechanical ventilation, it may affect between 8% and 38% of the patients who undergo this procedure. The mortality rates for these infections range from 24% to 76%[1] [15] [16][17].

Bloodstream infections (BSIs) were strongly associated with mortality (OR: 16.3252; p-value = 0.0122), which concurs with previous investigations into *P. aeruginosa* bacteremia[16] [18] [19]. The incidence of BSIs in patients who are hospitalized in ICUs ranges from 9% to 31%, and mortality rates vary according to symptom severity[8] [20].

Urinary tract infections were the third most common type of infection in the adult ICU, and the use of urinary catheters was a major risk factor for UTIs. These infections cause around 35-45% of HAIs[19]. The risk of these infections emerging depends on patient susceptibility and the levels of care exercised while

using urinary catheters. However, there are independent risk factors for UTIs that are associated with long-term urinary catheter use, including underlying diseases such as diabetes and immunodeficiencies[18] [21] [22].

An increase in the number of hospitalized patients who are infected with or colonized by gram-negative non-fermenting bacilli that display multidrug resistance characteristics has been observed over recent decades. This issue has received attention, particularly from personnel within infection control committees and health services that consider patients' clinical conditions and the variety of therapeutic approaches applied within health units[2] [19] [23].

From an epidemiologic perspective, undertaking prevalence studies and studies that characterize the risks associated with infectious diseases are essential, because these infections prolong hospitalizations and they can cause deaths[2] [15] [17] [24]. Such studies would provide sources of information about the main microorganisms circulating within hospitals and their antimicrobial resistance profiles [15] [17] [24].

This study involved adult, pediatric, and neonatal populations. Neonatal sepsis occurred in 73.7% of the neonates who were infected by *P. aeruginosa*; these were premature newborns mainly and the infections may have been associated with other factors, including maternal infections, particularly UTIs, prolonged hospitalizations, the use of broad-spectrum antibiotics, and invasive procedures, including central venous catheters and mechanical ventilation[22].

While the mortality rate was high in this study, it concurs with the findings from many other studies that have shown mortality rates that are up to six-times higher among patients who acquire MDRPA infections[15] [21] [22]. These findings suggest that death may have been mediated by more serious presentations of these infections or it may have been caused by underlying diseases, and they underline the need for adequate early antimicrobial therapy for these infections. The consequences of infections caused by *P. aeruginosa* are also reflected in the increase in the average length of the hospital stay. In this study, the average length of hospitalization was higher among the pediatric/neonatal patients.

We detected the presence of MDRPA strains and the *bla*SPM-1-*like* gene. Given the lack of data regarding the detection of this gene in northern Brazil, these findings suggest that continuous investigations are necessary within the hospitals and the region to monitor the presence of strains that may harbor this gene and its contributions to bacterial resistance and high mortality rates.

Interestingly, the MBL-producing isolates that were positive for the *bla*SPM-1-like gene were from two patients with BSIs, one patient with an RTI, and one patient with an UTI, and all progressed well and they were discharged. Hence, the expression of this gene may not necessarily be associated with the severity of the infection or the mortality of the patients who are infected by *P. aeruginosa* strains that produce this enzyme.

The molecular typing data demonstrated the presence of strains that were >97% similar genetically and they varied in relation to the resistance phenotypes that were present. Selective pressure in adult ICUs that is associated with inadequate empirical therapy may have caused these profile variations. However, despite the large quantities of antimicrobials used in the ICUs, only a small variation was identified in the neonatal ICU in this study.

The genetic differences among the *bla*SPM-1-like-positive isolates may indicate the presence of the gene on mobile elements. Likewise, the genetic clusters observed in the isolates that were positive or negative for the *bla*SPM-like-1 gene suggest horizontal gene acquisition, and we hypothesize that these genes are being carrried by resistance plasmids. Plasmid sequencing would improve our understanding of the genetic context of the *bla*SPM-1-like gene, but only a few known sequences exist that are adjacent to the gene[7].

All of the isolates had phenotypic resistance profiles that were similar to those reported by other investigators[24] [25] [26]. These data emphasize the need for epidemiologic investigations into this gene and periodic analyses of this pathogen's resistance profiles in the hospital that was studied to gain a better understanding of the evolution of *P. aeruginosa* resistance.

This study's findings show that invasive procedures and inadequate antibiotic therapy are significant factors that are associated with poor prognoses for patients who are infected by *P. aeruginosa* in ICUs. Although the mortality rate was high, multidrug resistance was not associated with death. The *bla*SPM-1-like gene was detected among the MDRPA strains, and this is the first time that this gene has been described in northern Brazil. Local monitoring of infections by MDRPA strains must be conducted to gain an understanding of this pathogen's resistance mechanisms and their impacts on public health. Alternative treatments for *P. aeruginosa* infections remain scarce; therefore, implementing and intensifying programs that promote the appropriate use of antibiotics are essential strategies to reduce the incidence of resistance.

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Conflict of interest

The authors declare that there is no conflict of interest.

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