Phenotypic and Genotypic Diversity of Multidrug-Resistant *Pseudomonas aeruginosa* Isolates from Bloodstream Infections Recovered in the Hospitals of Belo Horizonte, Brazil

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Key Words

Pseudomonas aeruginosa · Resistance markers · Genetic diversity · Bloodstream infections

Abstract

Background: *Pseudomonas aeruginosa* commonly causes nosocomial bloodstream infections and the emergence of a variety of β -lactamases (BLs) is worrying. In 5 hospitals in Belo Horizonte, Brazil, the presence of phenotypes encoding BL genes was established and the genetic diversity of the *P. aeruginosa* strains recovered from bloodstream infections was analyzed. **Materials and Methods:** The isolates were investigated using a disk diffusion (DD) method and the Etest[®], for encoding metallo- β -lactamases (MBLs), oxacillinases and cephalosporinases. Genes and genetic diversity were evaluated by random amplified polymorphic DNA (RAPD) genotyping and enterobacterial repetitive intergenic consensus (ERIC)-PCR. **Results:** Twelve strains (30%) were positive for MBLs by Etest and DD, 15 were cephalosporinase-positive and 87.5% were positive for *bla*_{SPM-1} and *bla*_{VIM-1}. Twenty-three strains (57.5%) were grouped into profile A, 32.5% into profile B and 10% into profile C by RAPD genotyping. ERIC-

PCR revealed a varying degree of similarity between strains, ranging from 45 to 100%. **Conclusions:** The results suggest distinct clonal populations in the 5 hospitals studied, indicating a potentially problematic epidemiological situation in Belo Horizonte, Brazil.

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Introduction

Pseudomonas aeruginosa is one of the major agents recovered from patients with nosocomial bloodstream infections. Its relevance is highlighted by the severity of these infections, its increasing drug resistance, the high costs of treatment and high morbidity [1].

The frequent use of antibiotics has contributed to the emergence of drug resistance and the production of a variety of β -lactamases (BLs) amongst *P. aeruginosa* strains, an important resistance mechanism observed in many bacteria [2]. Recently, multidrug resistance was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories [3]. The resistance of bacterial strains to several classes of antibiotics seriously

compromises the treatment of infected patients. In contrast to community-acquired strains, isolates from hospital environments exhibit high rates of antimicrobial resistance and are often resistant to multiple drugs [4]. *P. aeruginosa* bacteremia, in particular, is associated with high mortality. Reported mortality rates exceed 50% in some series and are even higher within certain populations, such as in patients with severe underlying comorbid conditions or those with immunosuppression [5].

P. aeruginosa populations undergo frequent recombination events contributing to the evolution of successful epidemic clones [6]. These populations are dominated by common clones that can be isolated from diverse clinical and environmental sources [7]. Thus, in order to genotype and differentiate between the isolates and clonal groups of *P. aeruginosa*, several molecular typing schemes have been proposed [8]. In this context, the establishing of accurate genetic similarity between individual genotypes is an essential and decisive point for clustering and analyzing inter- and intrapopulation diversity. The objective of this study was to evaluate the antimicrobial resistance and production of metallo- β -lactamase (MBL), oxacillinase and cephalosporinase enzymes, and the genetic diversity of strains of *P. aeruginosa* isolated from patients with bloodstream infections.

Material and Methods

Bacterial Identification and Drug Susceptibility Testing

Forty multidrug-resistant *P. aeruginosa* strains isolated from blood cultures between December 2008 and June 2009 were evaluated. All samples were previously identified using the GN card of the bioMérieux VITEK2[®] system. The strains were obtained from 5 hospitals that provide general assistance (clinical, surgical and hematological) and emergency services in the city of Belo Horizonte, Minas Gerais, Brazil.

Phenotypic screening for drug susceptibility was performed by the disk diffusion (DD) method, and minimum inhibitory concentration (MIC) determination by agar dilution (AD) with the following antimicrobials: 30 μ g ceftazidime (CAZ), 30 μ g cefepime (FEP), 30 μ g amikacin (AMK), 10 μ g gentamicin (GEN), 5 μ g ciprofloxacin (CIP), 5 μ g levofloxacin (LVX), 30 μ g aztreonam (ATM), 10 μ g imipenem (IMP), 100/10 μ g piperacillin/tazobactam (TZP), 10 μ g meropenem (MEM) and 300 μ g polymyxin B (PB). All records were obtained from Laborclin (São Paulo, Brazil). Results were interpreted according to the cut-off points recommended by the Clinical and Laboratory Standards Institute [9].

Phenotypic Detection of Class B (MBL) and Class C (Cephalosporinase) Enzymes Related to Resistance

To study MBLs, two different phenotyping methods were used: the DD method and the MBL Etest. DD was used to evaluate cephalosporinases.

MBL Evaluation

DD Method. This was performed with some modifications, as described below [10, 11]. The MBL inhibitors used in this study were 2-mercaptopropionic acid (MPA), 2-mercaptoacetic acid (MAA) and ethylenediaminetetraacetic acid (EDTA) (Sigma, St. Louis, Mo., USA) at concentrations of 2.11, 4.14 and 100 mM, respectively. The substrates used were disks containing 30 μ g CAZ and 10 μ g IMP (Laborclin, Pinhais, Brazil). Strains that exhibited antimicrobial synergism between CAZ and/or and at least one of the employed inhibitors were considered to be MBL-producing strains. MBL-producing samples displayed MBL distortion and a broadened zone of inhibition of bacterial growth in the region of diffusion of the MBL inhibitor. In negative tests, the zone of inhibition of bacterial growth was unchanged.

Etest[®]. Strips containing a gradient of IMP ranging from 4 to 256 μ g/ml at one end and from 1 to 64 μ g/ml of IMP associated with EDTA at the other end, were added to plates inoculated with the bacterial suspension and then incubated at 35°C for 24 h. A sample was considered to be MBL-producing when the ratio of the MIC (μ g/ml) for IMP to the MIC for IMP plus EDTA was ≥ 8 and when there was a decrease of 3 dilutions between IMP and IMP plus EDTA. The onset of deformation of the ellipse (i.e. the appearance of a ghost area) was also considered indicative of the production of MBLs and extended-spectrum BLs.

Cephalosporinase Evaluation

DD Method. This was performed with some modifications, as described below [9–11]. The bacterial suspension was prepared in saline for turbidity corresponding to point 0.5 on the McFarland scale, and this was inoculated with the aid of a swab on a 15-cm plate with agar Muller-Hinton medium (Becton-Dickinson[®], Le Pont de Blaix, France). A disk containing 30 μ g cefoxitin and 30 μ g CAZ (substrate) was placed with another disk containing 30 μ g ATM (inductor) at a distance of 2 cm. The appearance of a zone of ‘truncated’ junction between the 2 disks was considered presumptive for the presence of the extended-spectrum BL C Ambler class.

Genotypic Detection of BLs by PCR

Bacterial DNA was extracted by thermal lysis followed by centrifugation at 4°C for 30 s at 9,000 rpm [2, 7, 10]. The DNA content of the supernatant was quantified by Nanodrop[®]. Each reaction was performed in a final volume of 25 μ l with 12.5 μ l Master Mix (Promega Corp., Madison, Wis., USA) 1.5 μ l of primers (table 1) and 100 ng bacterial DNA plus nuclease-free water. PCR amplification was performed as previously described [8] to detect the following genes: *bla*_{SPM-1}, *bla*_{IMP-1}, *bla*_{GIM-1} and *bla*_{VIM-1}. For *bla*_{SIM-1} and *bla*_{AmpC}, the cycling conditions were as described by other authors [12, 13], respectively. For the *bla*_{OXA23}, *bla*_{OXA24}, *bla*_{OXA51} and *bla*_{OXA58} genes, PCR amplification was performed according to the literature [14].

Enterobacterial Repetitive Intergenic Consensus PCR

Enterobacterial repetitive intergenic consensus (ERIC)-PCR was used to rapidly type *P. aeruginosa* strains carrying the *bla*_{SPM-1} gene (35 strains). The ERIC1 (5'-ATGTAAGCTCCTGGGGATT-CAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') primers previously described were used [10, 15]. ERIC-PCR amplifications were performed with 100 ng of genomic bacterial DNA plus 12.5 μ l of Master Mix (Promega Corp.), 1.5 μ l of each primer and ultrapure water to bring the final reaction volume to 25 μ l. The

Table 1. Primers and expected product sizes used for detecting genotypic BLs

Gene	Primer sequence (5'–3')	Expected product size, bp	Reference No.
<i>bla</i> _{SPM-1}	AAAATCTGGGTACGCAAACG ACATTATCCGCTGGAACAGG	271	[7]
<i>bla</i> _{IMP-1}	GAATAGAGTGGCTTAACTCTC AGATAACCTAGTGGTTTGG	189	
<i>bla</i> _{VIM-1}	CGAATGCGCAGCACCAG CTGGTGCTGCGCATTCG	390	
<i>bla</i> _{GIM-1}	TCGACACACCTTGGTCTGAA AACTTCCAACCTTGCCATGC	477	
<i>bla</i> _{SIM-1}	TACAAGGGATTTCGGCATCG TAATGGCCTGTTCCTCATGTG	571	[8]
<i>bla</i> _{AmpC}	CAGTAGCGAGACTGCGCA ATAACCACCCAGTCACGC	631	[9, 13]
<i>bla</i> _{Oxa23}	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCTAT	501	[14]
<i>bla</i> _{Oxa24}	GGTTAGTTGGCCCCCTTAAA AGTTGAGCGAAAAGGGGATT	246	
<i>bla</i> _{Oxa51}	TAATGCTTTGATCGGCCTTG ATTTCTGACCGCATTTCCTAT	353	
<i>bla</i> _{Oxa58}	AAGTATTGGGGCTTGTGCTG CCCCTCTGCGCTCTACATAC	599	

PCR conditions included an initial denaturation at 95°C for 2 min followed by 30 cycles of 90°C for 30 s, 52°C for 1 min and 72°C for 1 min, with a final extension at 65°C for 8 min [10, 15].

All PCR-amplified products were resolved by electrophoresis on a 2% (w/v) agarose gel in Tris-acetate-EDTA buffer. The samples were run for approximately 120 min at 90 V. The specific products were observed after ethidium bromide staining using a standard ultraviolet-light transilluminator [10, 15, 16].

Random Amplified Polymorphic DNA Genotyping

The DNA of the all 40 strains of *P. aeruginosa* was subjected to random amplified polymorphic DNA (RAPD) genotyping with primers detecting *CagA*2, 5'-ATT TAG AAG CAG GCT TTA GC-3' and *CMVin*2, 5'-GGT AGC AC GCG GGT TTC GAC-3', which produce an average of 7–10 distinct PCR profiles per strain [17].

Gene amplification was performed with an initial cycle of 94°C for 2 min, followed by 5 cycles of denaturation at 94°C for 30 s, annealing at 30°C for 5 min and extension at 72°C for 1 min. This was then followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 30°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 7 min [17]. RAPD profiles were resolved by electrophoresis in a buffer (2 mM EDTA, 10 mM Tris-borate, pH 8.0) on a 6% polyacrylamide gel. The gel was silver-stained, visualized, photographed and analyzed, as described previously [18].

Quality Control

P. aeruginosa strains producing the enzymes of interest based on the phenotypic test were sequenced; after the presence of the corresponding gene had been confirmed, they were used as posi-

tive controls. As negative controls for all phenotypic tests, the reference strains *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used.

Ethical Aspects

This study was approved by the Research Ethics Committees of the participating hospitals and the COEP/UFGM (ETIC 614/08).

Data Analysis

The bands for each strain, primer and PCR method were scored as absent (0) or present (1) in order to construct a genotype dendrogram. To visualize hierarchical relationships among the sample profiles, the patterns generated by RAPD genotyping or ERIC-PCR were interpreted using numerical taxonomy and multivariate analysis (NTSYS v2.1, Exeter Software, New York, N.Y., USA). RAPD and ERIC-PCR dendrograms were constructed with the unweighted pair group method with arithmetic mean (UPGMA) using Dice's similarity coefficient.

Results

Antimicrobial Susceptibility Profiles

In this study, antimicrobial susceptibility testing of *P. aeruginosa* strains with the DD method and determination of MIC with the AD method showed high rates of resistance to all tested drugs. With the DD method, it was observed that 70–90% of the isolates were resistant to CAZ,

Fig. 1. Resistance profiles of 40 *P. aeruginosa* strains isolated from blood cultures from patients at 5 hospitals in this study (by DD).

Table 2. MIC₅₀ and MIC₉₀ of *P. aeruginosa* multiresistant strains (n = 40) isolated from blood cultures in 5 hospitals in Belo Horizonte

Anti-microbials	<i>P. aeruginosa</i>			MIC, µg/ml	
	breakpoints			MIC ₅₀	MIC ₉₀
	S	I	R		
IMP	≤4	16	≥32	4	64
MEM	≤8	16	≥32	2	16
CAZ	≤8	16	≥32	8	256
FEP	≤8	16	≥32	8	128
CIP	≤1	2	≥4	0.125	64
LVX	≤2	4	≥8	1	32
ATM	≤8	16	≥32	4	16
TZP	≤64/4	–	≥128/4	16	64
AMK	≤16	32	≥64	4	64
GEN	≤4	8	≥16	32	512
PB	≤2	4	≥8	0.25	0.5

MIC₉₀ = MIC capable of inhibiting the growth of 90% of the bacterial strains; MIC₅₀ = MIC capable of inhibiting the growth of 50% of the bacterial strains.

FEP, LVX, ATM, TZP, AMK and GEN and that 45.0% were resistant to IMP and MEM (fig. 1). Similar results were found with the AD method, with the MIC₉₀ (i.e. the MIC capable of inhibiting the growth of 90% of the bacterial strains) higher than the breakpoint for all antimicrobials tested and the MIC₅₀ higher for most of them (table 2).

Fig. 2. Detection of *bla*_{SPM-1} gene (271 bp) in *P. aeruginosa* isolates 9–19, 22 and 23. C+ = positive control. Molecular pattern: 50 bp.

Phenotypic Detection of BLs

Twelve (30%) *P. aeruginosa* strains were found to be positive for MBL with the Etest and DD methods. The percentage of positive samples ranged from 12.5 to 29.6%, according to each hospital. Regarding the DD method, the evaluated strains demonstrated synergy with at least one substrate and inhibitor. The CAZ + MPA, CAZ + MAA and CAZ + EDTA combinations were positive in 27.5% (11/40) of the strains and other combinations were positive in only 2.5% (table 3).

In the cephalosporinase (AmpC) phenotypic study, 15 (37.5%) *P. aeruginosa* strains were positive according to the DD method.

Fig. 3. Dendrogram based on Dice's coefficient of similarity using the UPGMA method implemented by the NT-SYS program, showing the relationships between 40 samples of *P. aeruginosa* isolated from hospitals A–E, and amplified by RAPD.

Genotypic Detection of BLs

Among the 40 strains of *P. aeruginosa*, 35 (87.5%) had PCR products corresponding to *bla*_{SPM-1} and *bla*_{VIM-1} gene fragments, and 35 (87.5%) of the amplified products were consistent with *bla*_{SPM-1} (fig. 2). The simultaneous occurrence of the *bla*_{SPM-1} and *bla*_{VIM-1} genes was observed in 6 strains of *P. aeruginosa* (15%). Genes *bla*_{IMP-1}, *bla*_{SIM-1}, *bla*_{GIM-1}, *bla*_{OXA23}, *bla*_{OXA24}, *bla*_{OXA58}, and *bla*_{AmpC} were not identified in any of the evaluated strains.

RAPD Genotyping

A dendrogram was constructed using RAPD data from 40 isolates. The hierarchical cluster analysis discriminated the genotypes of *P. aeruginosa* into 3 distinct profiles (A–C). Of the 40 DNA samples amplified, 23 (57.5%) were grouped into profile A, 13 (32.5%) into profile B and 4 (10%) into profile C (fig. 3). Table 4 shows the results of the RAPD genotyping of strains carrying the MBL genes and their respective units in the different profiles.

Table 3. Frequency of inhibitor-substrate combinations by phenotypic (DD method) for detection of MBLs in *P. aeruginosa* strains (n = 40)

Antimicrobial agent + inhibitor	Number of positive samples	%
IMP + MPA	1	2.5
IMP + MAA	1	2.5
IMP + EDTA	1	2.5
CAZ + MPA	11	27.5
CAZ + MAA	11	27.5
CAZ + EDTA	11	27.5

Enterobacterial Repetitive Intergenic Consensus

Genetic fingerprinting with ERIC primers produced consistent banding patterns for 35 *P. aeruginosa* strains carrying the *bla*_{SPM-1} gene, showing an average of 7–10 fragments per *P. aeruginosa* sample. These data generated

Table 4. RAPD genotyping profile of *P. aeruginosa* strains carrying genes for MBLs according to the hospital unit where patients were treated

Isolate	Hospital	Unit	Profile	Gene
1	A	ICU	A	<i>bla</i> _{SPM-1}
2		ICU	A	–
3		ICU	A	–
4	C	ICU	A	<i>bla</i> _{SPM-1}
5		ICU	A	<i>bla</i> _{SPM-1}
6		ONCO	A	<i>bla</i> _{SPM-1}
7		ICU	A	<i>bla</i> _{SPM-1}
8		ICU	A	<i>bla</i> _{SPM-1/VIM-1}
9		ICU	A	<i>bla</i> _{SPM-1}
10		ICU	A	<i>bla</i> _{SPM-1/VIM-1}
11		CLI	A	<i>bla</i> _{SPM-1}
12		CLI	C	<i>bla</i> _{SPM-1}
13	B	EXT	A	<i>bla</i> _{SPM-1}
14		ICU	A	<i>bla</i> _{SPM-1}
15		CLI	A	<i>bla</i> _{SPM-1}
16		CLI	A	<i>bla</i> _{SPM-1/VIM-1}
17	D	CLI	C	<i>bla</i> _{SPM-1}
18		CLI	A	<i>bla</i> _{SPM-1}
19		CLI	B	<i>bla</i> _{SPM-1}
20		CLI	B	–
21		CLI	B	–
22		CLI	B	<i>bla</i> _{SPM-1}
23		CLI	A	–
24	E	ICU	A	<i>bla</i> _{SPM-1}
25		ICU	B	<i>bla</i> _{SPM-1}
26		ICU	A	<i>bla</i> _{SPM-1/VIM-1}
27		CLI	A	<i>bla</i> _{SPM-1}
28		BTU	B	<i>bla</i> _{SPM-1}
29		BTU	C	<i>bla</i> _{SPM-1/VIM-1}
30		CLI	A	<i>bla</i> _{SPM-1}
31		CLI	C	<i>bla</i> _{SPM-1}
32		ICU	B	<i>bla</i> _{SPM-1}
33		BTU	B	<i>bla</i> _{SPM-1}
34		ICU	B	<i>bla</i> _{SPM-1/VIM-1}
35		CLI	B	<i>bla</i> _{SPM-1}
36		ICU	B	<i>bla</i> _{SPM-1}
37		BTU	B	<i>bla</i> _{SPM-1}
38		CLI	B	<i>bla</i> _{SPM-1}
39		ICU	A	<i>bla</i> _{SPM-1}
40		BTU	A	<i>bla</i> _{SPM-1}

BTU = Burn therapy unit; CLI = clinical unit; ONCO = oncology unit.

the matrix for the construction of the dendrogram from all samples. One high level of genetic diversity among the isolates was observed, demonstrating no genetic correlation between them (fig. 4).

Discussion

The increased use of carbapenems in hospitalized patients contributes to an increased selective pressure on nosocomial microorganisms, which favors the selection of bacterial subpopulations with reduced sensitivity or resistance to these antimicrobial agents. Bacterial strains of *P. aeruginosa* resistant to most antimicrobials and sensitive only to PB have been isolated by clinical microbiology laboratories in most Brazilian hospitals [19, 20]; this was corroborated by the results presented in this study. In a study over a period of 1 year in Mexico, *P. aeruginosa* was the third most frequently isolated species from blood in an intensive care unit (ICU) and the predominant isolate in central venous catheters among the species included in the ESKAPE group of multidrug-resistant microorganisms [21].

The production of MBL enzymes by some pathogen types is of concern worldwide. These enzymes are notable for their broad spectrum of activity against most β -lactam agents, including carbapenems, and also their resistance to BL inhibitors [22]. MBLs hydrolyze all the β -lactam drugs that are commercially available, with the monobactams, e.g. ATM, being the only exception [2, 22, 23]. ATM shows antimicrobial activity in vitro against *P. aeruginosa* strains, with a sensitivity rate of 67.5%. This profile is consistent with the production of MBLs by these strains because ATM is not hydrolyzed by these enzymes. However, the results of our study showed that 32.5% of *P. aeruginosa* strains were resistant to ATM; this suggests a possible association with other resistance mechanisms, or even the production of BLs.

P. aeruginosa-producing MBLs are often resistant to all treatment options, so drugs such as PB are frequently the therapeutic choice, despite their toxicity [22, 24]. In our study, 5% (2/40) of *P. aeruginosa* strains were intermediately resistant to PB, and the MIC₅₀ and MIC₉₀ for this antimicrobial were 0.25 and 0.5 mg/ml, respectively.

In the phenotypic MBL research, a higher number of producing samples was observed when EDTA was used as inducer. It is known that EDTA may increase bacterial cell wall permeability and that zinc (chelated by EDTA) accelerates decomposition and IMP decreases OprD expression of *P. aeruginosa* [25].

In this study, due to the higher sensitivity and specificity of PCR, we used this methodology for the detection of MBLs, but the phenotypic expression levels of MBL genes were not assessed. The differences observed here between the phenotypic and genotypic methods of detection of

Fig. 4. Dendrogram based on Dice's coefficient of similarity using the UPGMA method implemented by the NTSYS program, showing the relationships between 35 samples of *P. aeruginosa* isolated from hospitals A–E, and amplified by ERIC-PCR.

MBLs may be because MBL producers might act as silent reservoirs of such resistance determinants [26].

Different DNA typing methods have been frequently used to investigate the diversity of antibiotic resistance genes and the epidemiological distribution of *P. aeruginosa* [8–27]. In this study, PCR assays were used to evaluate the presence of carbapenem-hydrolyzing class D BL and MBL genes, while ERIC-PCR and RAPD genotyping were performed to identify and establish the relatedness between the isolates. PCR is an appropriate method for the detection of different reference genes encoding BLs [8–16, 27]. Brazil, which has a significant continental land mass and is the largest country in Latin America, is characterized by wide variations in geography and economy. By means of PCR assays, several MBL genes in *P. aeruginosa* have been detected in different regions of Brazil. One study in Uberlândia, Minas Gerais, detected the *bla*_{SPM-1} gene in 33% (3/15) of the samples [28] while in Passo Fundo, Rio Grande do Sul, this gene was detected in 77.6% (173/223) [20] of the samples. In São Paulo, São Paulo, another 2 studies detected the gene *bla*_{SPM-1}; the first was detected in 17.2% (n = 5/25) [29] of the isolates, and the second reported *bla*_{SPM-1} in 81% and *bla*_{VIM-2} in 19% of the isolates [30]. In our study, the genotypic detection of BLs showed that 87.5% (35/40) of *P. aeruginosa* samples carried the *bla*_{SPM-1} gene and 15% (6/40) carried the *bla*_{VIM-1} gene.

Using RAPD genotyping to evaluate 40 *P. aeruginosa* strains, it was possible to identify 3 different genetic profiles: 23 (57.5%) strains with profile A, 13 (32.5%) strains with profile B and 4 (10%) strains with profile C. According to the data generated by the similarity analysis, strain MG1 (from the ICU of hospital A) and strains MG13 and MG14 (from the ICU of hospital C) showed the same profile bands (fragments of 150, 165, 195, 280 and 330 bp), with a similarity of approximately 85%. The same similarity was observed for fragments of 165, 195, 280, 330 and 450 bp for samples MG2, MG11 and MG8 from hospital B.

As shown in the dendrogram, Dice's coefficient measures suggest that there is a clonal population with the same lineage of resistance genes from different sectors in the same hospital as well across institutions. It is noteworthy that all these strains carried the *bla*_{SPM-1} gene and the strain MG8 carried the *bla*_{SPM-1} and *bla*_{VIM-1} genes simultaneously.

Conclusions

With the DD method and MIC determination, we evaluated bacterial resistance to antimicrobials and revealed high rates of antimicrobial resistance among *P. aeruginosa* strains. Even resistance to PB, which is considered quite effective against the bacteria studied, was ob-

served. Genes encoding MBL (i.e. *bla*_{VIM} and *bla*_{SPM}) have been identified in almost all *P. aeruginosa* strains evaluated, while those responsible for the production of oxacillinases (*bla*_{OXA23}, *bla*_{OXA24}, *bla*_{OXA51} and *bla*_{OXA58}) were identified only in some of them. Finally, our results suggest that certain clonal populations are circulating among representative strains in the different hospitals evaluated, demonstrating a worrying epidemiological situation in the city of Belo Horizonte. Further studies with a larger number of strains would be required to confirm and obtain a better characterization of the panorama of resistance in the hospitals studied. Healthcare professionals must be alert and follow standard and/or specific precautions to avoid dissemination inside each hospital and between different hospitals.

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Disclosure Statement

There are no conflicts of interest.

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