Carbapenem-resistant \textit{Pseudomonas aeruginosa} strains: a worrying health problem in intensive care units

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ABSTRACT

\textit{Pseudomonas aeruginosa} is one of the most common bacterium with a broad spectrum of human-associated infections. It is intrinsically resistant to many antimicrobial drugs, making carbapenems crucial in clinical management. The emergence and dissemination of carbapenemases among \textit{P. aeruginosa} clinical isolates is a serious public health concern as it limits the options for the treatment of bacterial infections. Here, we described the molecular and epidemiological characteristics of 28 carbapenem-resistant \textit{P. aeruginosa} strains isolated from patients hospitalized in an intensive care unit (ICU). The antimicrobial susceptibility of carbapenem-resistant \textit{P. aeruginosa} strains was determined by broth microdilution. The presence of resistance genes was evaluated by PCR and DNA sequencing. Additionally, alterations in genes encoding \textit{P. aeruginosa} outer membrane proteins were analyzed by PCR as well as SDS-PAGE. Clinical characteristics of the patients and the economic impact of hospitalization on the public health system were evaluated. PCR amplification showed that the \textit{bla\textsubscript{KPC-2}} and \textit{bla\textsubscript{TEM}} genes were identified in three isolates (11\%) and \textit{bla\textsubscript{SHV}} gene in two isolates (7\%). Outer membrane profiles obtained by SDS–PAGE indicated that the \textit{OprD} porin was either absent or was produced at very low levels. A PCR assay using \textit{oprD}-specific primers failed to show the presence of mutations in this gene. \textit{P. aeruginosa} strains were isolated from 28 patients, among whom 43\% (12/28) had sepsis, 31\% (9/28) had respiratory failure, and 31\% (9/28) had systemic arterial hypertension. A high mortality rate (39\%) was observed in these patients, with an average duration of hospitalization of 34.6 days and a median cost of 3.275 dollars per patient. The production of carbapenemase was not the main mechanism of resistance in these strains. All carbapenem-resistant \textit{P. aeruginosa} were isolated from patients hospitalized in the ICU. Besides the high mortality rate, many patients remained hospitalized for several days, resulting in a high cost of hospitalization for the public health system. Therefore, the evolution of this resistance and its dissemination should be actively monitored among critically ill patients to improve their health conditions.

KEYWORDS: Healthcare-associated infections. Antimicrobial resistance. Public health. Carbapenemase.

INTRODUCTION

\textit{Pseudomonas aeruginosa} is a common cause of serious healthcare-associated problems, usually causing urinary, respiratory, and bloodstream infections\textsuperscript{1}. These infections can be fatal for critically ill and immunocompromised patients and are further exacerbated by antimicrobial resistance\textsuperscript{2}. There is some evidence suggesting that patients who are infected by carbapenem-resistant pathogens have an increased likelihood of morbidity and mortality compared to those infected by susceptible
pathogens\textsuperscript{3,4}. This is probably due to the administration of antibiotics with suboptimal or no effect against these organisms\textsuperscript{5}. The resistance to carbapenem in Gram-negative bacteria has become a global problem, which has driven the World Health Organization (WHO) to include carbapenem-resistant \textit{P. aeruginosa} in the list of pathogens of high priority for the research and development of new antibiotics. According to the Centers for Disease Control and Prevention (CDC), an estimated 51,000 healthcare-associated \textit{P. aeruginosa} infections in US hospitals occur annually. More than 6,000 (13\%) of these are multidrug-resistant, with about 440 deaths per year\textsuperscript{6}.

In Brazil, the rate of multidrug-resistant \textit{P. aeruginosa} in adult and neonatal ICUs increased from 35.6\% to 39.1\% and 18.2\% to 29.3\%, respectively\textsuperscript{7}. The impact of the resistance of \textit{P. aeruginosa} on health systems is a major concern and it has spread across hospitals mainly due to the frequent use of carbapenems, which has been considered the only effective antibiotic against \textit{P. aeruginosa} infections\textsuperscript{3}. Recognizing the risk of resistance to carbapenem especially in the most vulnerable patients and/or the early detection of specific carbapenem-resistance mechanisms are critical to reducing the risk of mortality, length of hospitalization, and associated costs\textsuperscript{3,9}. To achieve this, identification and ongoing surveillance of carbapenem-resistant Gram-negative bacteria are needed. Here, we described the epidemiological and molecular characteristics of carbapenem-resistant \textit{P. aeruginosa} strains isolated from patients hospitalized in ICUs.

**MATERIALS AND METHODS**

**Study site and patients**

Data were collected from patients hospitalized in a public tertiary care hospital in the municipality of Dourados, Mato Grosso do Sul State, Midwest Brazil, between November 2015 and August 2016. The hospital has 237 beds, distributed across the infirmaries and the ICUs (adult, pediatric, and neonatal). The hospital serves as a tertiary referral center for 32 cities, with an average of 9,800 annual admissions. The majority of the patients lived in Dourados, while the remaining lived in the surrounding cities.

**Bacterial identification and susceptibility testing**

The identification of the bacterial species and the screening for antimicrobial resistance were performed by the Phoenix\textsuperscript{\textregistered} Automated System (BD Diagnostic Systems, Sparks, MD, USA) according to the manufacturer’s instructions. After isolation, the susceptibility profile was confirmed, and the minimum inhibitory concentrations (MICs) of the antimicrobials were determined by broth microdilution following the recommendations of the Clinical and Laboratory Standards Institute guidelines (CLSI)\textsuperscript{10}.

**PCR for resistance genes**

Carbapenemase encoding genes were investigated by the polymerase chain reaction (PCR) and DNA sequencing\textsuperscript{11}, using specific primers for: (I) serino-\(\beta\)-lactamasas (\textit{bla}_{\text{TIM}}, \textit{bla}_{\text{SHV}}, \textit{bla}_{\text{GES}}, \textit{bla}_{\text{CTX-M}}, \textit{bla}_{\text{HES}}, \textit{bla}_{\text{PER}}, \textit{bla}_{\text{KPC}}); (II) oxacillinases (\textit{bla}_{\text{OXA-23}}, \textit{bla}_{\text{OXA-24}}, \textit{bla}_{\text{OXA-48}}, \textit{bla}_{\text{OXA-51}}, \textit{bla}_{\text{OXA-58}}); and (III) metallo-\(\beta\)-lactamasases (\textit{bla}_{\text{IMP}}, \textit{bla}_{\text{VIM}}, \textit{bla}_{\text{SPM}}, \textit{bla}_{\text{GIM}}, \textit{bla}_{\text{SIM}}, \textit{bla}_{\text{NDM}}). The PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and both strands were sequenced using the Applied Biosystems 3500 genetic analyzer equipment (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The nucleotide sequences and the deduced amino acid sequences were analyzed using the Lasergene software package (DNAStar, Madison, WI, USA) and compared with the sequences available on the Internet using the BLAST tool.

**Porin expression and \textit{oprD} mutations**

Mutations in \textit{OprD} and its promoter region were identified by conventional PCR, and the amplicon size was analyzed by a 1.5\% agarose gel electrophoresis\textsuperscript{12}. The outer membrane proteins (OMPs) were isolated and visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using membrane extracts from bacteria grown in nutrient broth overnight, following which, the gels were stained with Coomassie blue. \textit{Pseudomonas aeruginosa} ATCC 27853 was used as a reference strain.

**Clinical data**

The following data of all the patients participating in this study were recorded: demographics; location before admission; hospital course (duration); comorbidities, treatment regimens, source of infection, and outcome (recovery/death) (Table 1). Strict CDC definitions were used to determine whether an isolated organism was associated with colonization or infection. The presence of a clinical infection was determined by the medical diagnosis, and the decision to initiate antimicrobial therapy, as well as the isolation of a carbapenem-resistant \textit{P. aeruginosa} strains, according to the following clinical criteria (sepsis,
| Patient | Gender | Age (years) | Clinical specimen  | Lenght of stay (days) | Comorbidities | HAI | Outcome | Treatment |
|---------|--------|------------|--------------------|----------------------|---------------|-----|---------|-----------|
| 1       | M      | 47         | Tracheal aspirates | ND                   | ND            | C   | ND      | ND        |
| 2       | M      | 80         | Tracheal aspirates | 30                   | SAH, SEP, CARD | I   | Death   | MTZ/1500 mg/5 d PIP+TAZ/13.5 g/ 5 d MEM/3000 mg/ 6/day 2000 mg/ 18 d VANCOCO/2000 mg/ 6/day 1000 mg/7 d PMB/500 mg/ 17 d TGC/200 mg/11 d |
| 3       | M      | 37         | Urine culture      | 38                   | DM            | I   | Death   | CRO/2000 mg/4 d LVX/750 mg/ 2 d PIP+TAZ/ 4 g+ 0.5 g/ 3 d PMB/ 500 mg/ 20 d TEICO/ 800 mg/ 3 d TGC/100 mg/ 20 d MEM 3 g/26 d/ 3 g/ 5 d |
| 4       | M      | 61         | Tracheal aspirates | 92                   | NEURO, CARD PNA | C   | Recovery | MTZ/500 mg/13 d MEM/500 mg/ 17 d TEICO/ 400 mg/3 d/ 200 mg/14 d |
| 5       | M      | 17         | Tracheal aspirates | 36                   | CARD, NEURO, SEP | C   | Recovery | AMPSAM/1 g+ 0.5 g/ 4 d CLI/ 1200mg/5 d MEM/3 g/ 26d VAN/ 2 g/36 d PMB/ 500 mg/ 2 2d TGC/100 mg/ 1 d/50 mg/2 2d |
| 6       | F      | 45         | Tracheal aspirates | ND                   | ND            | C   | ND      | ND        |
| 7       | F      | 54         | Tracheal aspirates | 42                   | DM, SAH, CARD, SEP | C   | Death   | MEM/3 g/26 d VANC/ 2 g/ 8 d PMB/ 500 mg/ 26 d AMP-TAZ/1 g +0.5 g/ 1 d LZD/ 1200 mg/ 3 d TEICO/ 800 mg/ 5 d IMP+CIS/ 2 g/ 4 d TGC/ 100 mg/15 d |
| 8       | M      | 56         | Blood culture      | 16                   | ALCOH, SAH, CANS, TAB, HEMAT, SEP | Death | IMP + C/2 g/4 d FEP/2 g/ 7 d MEM/6 g/5 d VANC/ 3 g/1 d/ 2 g/ 1 d |
| 9       | M      | 84         | Catheter           | 48                   | HEMAT, HYPER  | C   | Death   | MEM/ 3 g/14 d/3 g/19 d TEICO/ 800 mg/ 1 d/800 mg/6 d VANC/500 mg/3 d TGC/100 mg/29 d PMB/ 500 mg/ 17 d IMP+CIS/ 2 g/6 d |
| 10      | F      | 24         | Secretion culture  | 44                   | RESP, SEP     | C   | Death   | MEM/ 1 g/28 d/3 g/14 d/3 g/19 d VANC/500 mg/3 d TGC/100 mg/29 d PMB/ 500 mg/ 17 d IMP+CIS/ 2 g/6 d |
| 11      | F      | 26         | Urine culture      | ND                   | NEURO, SEP    | I   | Recovery | MEM/ 1 g/28 d/3 g/14 d/3 g/19 d VANC/500 mg/3 d TGC/100 mg/29 d PMB/ 500 mg/ 17 d IMP+CIS/ 2 g/6 d |
| 12      | F      | 34         | Urine culture      | ND                   | ND            | C   | ND      | ND        |
| 13      | F      | 14         | Tracheal aspirates | 15                   | RESP, NEURO, PNA | C   | Recovery | PIP+TAZ/ 4g+0.5 g/10 d AMK/ 500 mg/ 3 d |
| 14      | M      | 35         | Tracheal aspirates | 15                   | RESP          | I   | Recovery | AMOX+CLA/ 500 mg/100 mg/15 d |
| 15      | F      | 65         | Urine culture      | 30                   | ND            | I   | Recovery | IMP+CIS/500 mg/500 mg/24 d TEICO/400 mg/7 d PMB/ 50 mg/ 20 d |
### Table 1 - Clinical characteristics of 28 patients infected/colonized with carbapenem-resistant *Pseudomonas aeruginosa* strains. (cont)

| Patient | Gender | Age (years) | Clinical specimen | Length of stay (days) | Comorbidities | HAI | Outcome | Treatment |
|---------|--------|-------------|-------------------|-----------------------|---------------|-----|---------|-----------|
| 16      | F      | 70          | Tracheal aspirates | 38                    | DM, DEC, SAH, SEP | C   | Death   | MEM/ 3,000 mg/1 d/2,000 mg/6 d/2,000mg/14 d IMP+CIS/ 500 mg-500mg/ 17 d VAN/2,000 mg/14 d GEN/80 mg/ 14 d PMB/ 50 mg/ 14 d |
| 17      | M      | 79          | Urine culture     | 40                    | ALCOH, SAH, COPD, CARD, TAB, NEURO, SEP | I   | Death   | CRO 200 mg/ 5 d PIP+TAZ/ 4g-0.5g/3 d MEM/ 3 g/2 d/15 g/5 d VAN 2 g/16 d IMI+CIS/500 mg-500 mg/15 d |
| 18      | M      | 81          | Tracheal aspirates | 30                    | ND            | C   | Recovery | AMP-SAM/1g+0.5 g/7 d |
| 19      | M      | 76          | Urine culture     | 23                    | SAH, CARD, REN, | C   | Death   | ND |
| 20      | M      | 35          | Tracheal aspirates | 38                    | RESP, NEURO, SEP | C   | Recovery | CLI/ 150 mg/mL/ 7 d TIZP/4 g-0.5 g/ 5 d TEICO/800 mg/2 d/ 400 mg/6 d PMB/ 50 mg/22 d SXT+ TMP/40 mL/ 3 d|
| 21      | M      | 76          | Tracheal aspirates | 25                    | PNA           | I   | Death   | GEN/80 mg/ 7 d PMB/50 mg/ 14 d TEICO/400 mg/ 7 d TGC/80 mg/ 7 d MEM/3 g/ 7 d/6 g/ 12 d CIP/1,200 mg/1 d/800 mg/11 d SXT+ TMP/80 mL/ 7 d |
| 22      | F      | 28          | Urine culture     | 30                    | NEURO, SEP    | C   | Recovery | GEN/80 mg/7 d |
| 23      | M      | 51          | Secretion culture | 13                    | DM, SAH, RESP | C   | Recovery | MEM/ 3 g/2 d LZD/1,200 mg/1 d PMB/50 mg/ 1 d |
| 24      | F      | 21          | Urine culture     | 30                    | RESP, SEP     | I   | Recovery | MEM/ 3 g/ 4 d PMB/50 mg/16 d GEN/80 mg/ 16 d |
| 25      | M      | 81          | Secretion culture | 28                    | RESP, NEURO   | C   | Recovery | AMP-SAM/1 g +0.5 g/11 d PMB/50 mg/ 15 d |
| 26      | M      | 51          | Secretion culture | 30                    | RESP, SAH, CARD | C   | Death   | VAN 1 g/ 21 d MEM 1 g/ 21 d |
| 27      | F      | 48          | Secretion culture | 39                    | SAH, SEP      | C   | Recovery | CRO/2,000 mg/ 8 d MTZ/2,000 mg/ 7 d IMP+CIS/ 500 mg/12 d PMB/50 mg/14 d TEICO/800 mg/3 d/400 mg/ 9 d GEN/80 mg/ 5 d MEM/3,000 mg/1 d/2000 mg/11 d |
| 28      | F      | 1           | Secretion culture | 30                    | RESP, PNA     | C   | Recovery | CFE/3 mL/4 d/ 4 mL/8 d |

Treatments: Amikacin (AMK), Amoxicillin(AMOX), Clavulanatepotassium (CLAV), Ciprofloxacin (CIP), Gentamycin (GEN), imipenen (IMP), levofloxacin (LEV), meropenem (MEM), Piperacilin (PIP), Polimixin B (PMB), Tigecilin (TGC), Ampicilin + sulbactam (AMP-SAM), cefalexin ( CFE), Teicoplamin (TEICO), Metronidazole (MTZ), sulfametoxazole+ trimetropim (SXT-TMP), Vancomycin (VAN), Cilastatin (CIS), Linezolid (LZD), ceftriaxone (CRO), Clindamicin (CLI), Levofloxacin (LVX), Tazobactam (TAZ); Comorbidities: Alcoholism (ALCOH), Cancer (CANC), Cardiovascular disease (CARD), Chronic Obstructive Pulmonary disease (COPD), Decubitus ulcers (DEC), Diabetes Mellitus (DM), Hyperthyroidism (HYPER), Neurological disease (NEURO), Pneumonia (PNA), Renal Failure (REN), Respiratory Failure (RESP), Systemic Arterial Hypertension (SAH), Sepsis (SEP), Tabagism (TAB); Healthcare-associated Infections (HAI); Infection (I), Colonization (C); ND = not detected.
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fever, changes in the frequency or color of secretions, or new radiological findings). Colonizers were defined as bacteria permanently or temporarily present on the skin or the mucous membranes of the patients, irrespective of the signs or symptoms of infection. The endemic level of colonization and infection by carbapenem-resistant strains per 1,000 patients-days was calculated using a previously described method. The costs of hospitalization of the patients in ICUs were estimated using data from the Unified Health System (SUS) of Brazil. We consulted the average daily cost in the ICU by specialty, in the municipality of Dourados, Mato Grosso do Sul State, in December-2020, through the official database of Analytical Audit in SUS Hospitalizations (DATASUS).

Ethical standards

This study was conducted with the approval of the Research Ethics Committee from Universidade Federal da Grande Dourados (Nº 877.292/2014).

RESULTS

Antimicrobial susceptibility testing

Twenty-eight carbapenem-resistant *P. aeruginosa* strains were isolated from patients hospitalized in an ICU. All the strains exhibited resistance to IMP and MEM with MICs ranging from 32 to ≥ 128 µg/mL and from 8 to ≥128 µg/mL, respectively. This resistance was observed in 75% (21/28) of the isolates for ATM (MIC range: ≥ 8 to 16 µg/mL), 68% (19/28) for GEN (MIC range: ≥ 2 to 8 µg/mL), 60% (17/28) for CAZ (MIC range: ≥ 2 to 16 µg/mL), 71% (20/28) for FEP (MIC range ≥ 2 to 16 µg/mL), 71% (20/28) for PIP (MIC range ≤ 4 to ≥ 64 µg/mL), 71% (20/28) for CIP (MIC range ≤ 0.5 to ≥ 2 µg/mL), 53% (15/28) for AMK (MIC range ≤ 8 to ≥ 32 µg/mL), 3.5% (01/28) for CST (MIC range ≤ 1 to ≥ 4 µg/mL), and 68% (19/28) for LEV (MIC range ≤ 2 to ≥ 4 µg/mL). Individual results are described in the Supplementary Table S1.

Molecular characterization

The *bla*<sub>TEM</sub> gene was detected in 11% (3/28) of the carbapenem-resistant *P. aeruginosa* strains, *bla*<sub>KPC-2</sub> in 11% (3/28), and *bla*<sub>SHP</sub> in 7% (2/28) of the resistant strains (Table 1). The *bla*<sub>oxa-4</sub>, *bla*<sub>oxa-5</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>GES-5</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>IMP</sub>, *bla*<sub>EKO</sub>, *bla*<sub>SPM</sub>, *bla*<sub>IMI</sub>, and *bla*<sub>MCR</sub> genes were not amplified. The PCR analysis of the *oprD* porin gene failed to amplify all the isolates (100%) (S1). According to the SDS–PAGE results, OprD was not identified, suggesting that OprD was either absent or was produced at very low levels by all isolates (Table 1).

Clinical data

The carbapenem-resistant *P. aeruginosa* strains were isolated from 28 patients hospitalized in ICUs, whose median duration of stay was 34.4 days. Previously, 75% (21/28) of the patients were hospitalized in another hospital. Out of the 28 patients, 43% (n = 12/28) were female, their ages ranging from 1 to 84 years (median 48.9). Regarding the history of comorbidities, 43% (12/28) of the patients had sepsis, 31% (9/28) had respiratory failure, 31% (9/28) had systemic arterial hypertension, 28.5% (8/28) had neurological diseases, 25% (7/28) had cardiovascular diseases, 14% (4/28) had pneumonia, 14% (4/28) had diabetes mellitus, 7% (2/28) had a smoking history, and 7% (2/28) were alcoholic (Table 1). Carbapenem-resistant *P. aeruginosa* strains were recovered from tracheal secretions in 43% (12/28) of the patients, from urine in 28.5% (08/28) of the patients, from secretions culture in 21% (06/28) of the patients, from blood cultures in 3.5% (01/28) of the patients, and from intravenous catheters in 3.5% (01/28) of the patients. Among the carbapenem-resistant strains, 28.5% (8/28) were considered true pathogens (I) and 71.5% (20/28) were colonizers (C) (Table 1). The three most common treatment regimens used in the patients were: carbapenems (meropenem, imipenem) in 89% (25/28), polypeptides (polymyxin B) in 82% (23/28), and glycopeptides (teicoplanin, vancomycin) in 53.5% (15/28). The mortality rate of patients infected or colonized with carbapenem-resistant *P. aeruginosa* was 39% (11/28).

Regarding the endemicity of carbapenem-resistant *P. aeruginosa* strains, the rate of occurrence of infection and/or colonization per 1,000 patient-days in June 2016 exceeded the average incidence of colonization/infection, as per the established alert limit (Figure 1). The acquisition rate of infection and/or colonization was 0.01 per 1,000 patient-days in May 2016, increasing to 0.03 per 1,000 patient-days in June 2016. Although initially it did not exceed the control limit, subsequent cases led the acquisition rate of carbapenem-resistant strains to rise above the mean prevalence, causing it to reach the alert limit at different times of the study period. Patients infected or colonized with carbapenem-resistant strains remained hospitalized for a minimum of 13 days. Each patient infected or colonized with carbapenem-resistant strains spent a median of US$ 3,174.87 in the treatment (Brazilian reals were converted to U.S. dollars using the quotation of December 21, 2020 when R$ 5.22 = US$ 1.00, resulting in US$ 91.70 per day of hospitalization.
DISCUSSION

The prevalence of antimicrobial resistance is increasing, probably due to the widespread use of carbapenems in the hospital environment. This can selectively increase the pressure on the hospital microbiota, in turn, increasing the emergence and spread of resistant bacteria; thus, affecting the morbidity and mortality of the patients. The ICUs are the major sources of dissemination of multidrug-resistant organisms, where the selection pressure is the highest for the emergence of resistance. The nosocomial infection rate in ICUs is 2–5 times higher than that in the general (non-ICU) hospital population. Interestingly, in our study, all carbapenem-resistant *P. aeruginosa* strains were isolated from patients hospitalized in the ICU, indicating that in this hospital, the frequency of these strains is restricted to the ICU.

Out of the 28 carbapenem-resistant *P. aeruginosa* strains evaluated, only three KPC-producing *P. aeruginosa* strains were identified. The production of carbapenemase has emerged in Brazil as the main mechanism of resistance to carbapenem among clinical isolates of *P. aeruginosa*. However, the low rates of KPC-producing *P. aeruginosa* observed suggest that other mechanisms could be involved in the evolution of resistance. In our study, OprD porin was absent or was produced at very low levels in carbapenem-resistant *P. aeruginosa*, indicating that the loss of OprD has contributed to carbapenem resistance. Sequence changes including amino acid deletions or substitutions, probably led to an increase in the minimum inhibitory concentration of carbapenems. The emergence of resistance to *P. aeruginosa* to carbapenems associated with the loss of porin by an insertion sequence element has been associated with an increase in the MIC of imipenem and meropenem from 0.5 and 2 µg/mL, respectively, to up to 16 µg/mL. Several studies have reported that mutations, insertions, and/or deletions are responsible for inactivating the oprD gene, therefore conferring resistance to imipenem and meropenem in clinical isolates of *P. aeruginosa* recovered worldwide. Thus, the deficiency of the outer membrane protein OprD appears to be the mechanism involved in the resistance of *P. aeruginosa* strains evaluated in our study. Several mechanisms are involved in the drug resistance of *P. aeruginosa*.

The carbapenem-resistant *P. aeruginosa* strains were isolated from patients hospitalized in ICUs for long periods (mean 34.4 days). They had several comorbidities and poor outcomes (39% mortality rate). The emergence of resistance during treatment is associated with increased morbidity, mortality, and health costs. *Pseudomonas aeruginosa* is one of the main pathogens in ICUs; responsible for high mortality rates, ranging from 24.8% in patients infected with sensitive *P. aeruginosa* to 44.6% in patients infected with multidrug-resistant *P. aeruginosa*. The acquisition of carbapenem-resistance in *P. aeruginosa* strains plays an important role in the high mortality rates. However, they
are not the only factor responsible for the poor outcomes observed in the studied patients, considering that they displayed several other unfavorable clinical conditions\textsuperscript{35-37}.

Besides having negatively impacted the clinical outcomes, carbapenem resistance has also created an undue economic burden with a high cost of hospitalization (median cost of US$ 3,174,87, for 34.61 days) as we have demonstrated here. Previous studies showed that carbapenem resistance was a significant predictor of in-hospital mortality\textsuperscript{38} and resulted in an increase in hospital-related costs\textsuperscript{37}. The resistance of \textit{P. aeruginosa} to antimicrobials is a global problem, especially in countries with limited resources, due to the need for new treatment options and improvement of basic practices for the prevention and surveillance of infections\textsuperscript{39}. In hospitals, an active surveillance for the early detection of colonized patients is necessary to prevent institutional outbreaks and to limit dissemination; the early intervention to prevent death through the administration of antimicrobials is effective in controlling the threat\textsuperscript{40}.

There are, however, some limitations in our study. Firstly, it was performed in only one hospital. Thus, the prevalence and molecular characteristics of carbapenem-resistant \textit{P. aeruginosa} strains may not be generalizable across the country. In addition, we had several difficulties in determining the clinical characteristics of some patients from whom the carbapenem-resistant \textit{P. aeruginosa} strains were isolated, so that they were excluded from the study. Excluding the limitations, our study added important data to the literature by showing that patients hospitalized in ICUs are primarily affected by carbapenem-resistant \textit{P. aeruginosa} strains. Additionally, the production of carbapenemases may not be the main mechanism of resistance in \textit{P. aeruginosa} strains circulating in the hospital.

Our results showed that alterations in OprD may be responsible for the resistance to carbapenem among \textit{P. aeruginosa} isolated from patients hospitalized in Dourados, Brazil. Identifying the factors that can influence the mobilization and insertion of elements that disrupt the \textit{oprD} gene can contribute significantly to our current knowledge of mechanisms involved in the emergence of antibiotic resistance and the evolution of \textit{P. aeruginosa}. Additionally, stricter measures to contain the spread of multi-resistant microorganisms can contribute to the reduction of mortality of patients hospitalized in ICUs, as well as to reduce the costs of hospitalization.

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**CONFLICT OF INTERESTS**

The authors have declared that no competing interests exist.

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