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Article

# High genetic diversity among *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated in a public hospital in Brazil

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In Brazil and other regions of the world, *Pseudomonas aeruginosa* and *Acinetobacter* spp. have emerged as important agents of nosocomial infection and are commonly involved in outbreaks. The main objective of the present study was to evaluate the genetic relationship among *P. aeruginosa* and *Acinetobacter* spp. isolated from patients in a public university hospital in northwestern Paraná, Brazil, and report their antimicrobial resistance profile. A total of 75 *P. aeruginosa* and 94 *Acinetobacter* spp. isolates were phenotypically identified and tested for antibiotic susceptibility using automated methodology. Polymyxin B was tested by disk diffusion for *P. aeruginosa*. Metallo-β-lactamase (MBL) was detected using a disk approximation test. Genotyping was performed using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). Approximately 55% of the *P. aeruginosa* isolates and 92% of the *Acinetobacter* spp. isolates were multiresistant, but none were MBL-producers. ERIC-PCR revealed the presence of small clusters of carbapenem-resistant *Acinetobacter* spp., most likely OXA-type carbapenemase producers. Furthermore, high genetic diversity in *P. aeruginosa* and *Acinetobacter* spp. clinical isolates was observed, suggesting that cross-transmission is not very frequent in the studied hospital.

**Uniterms**: *Pseudomonas aeruginosa/*antimicrobial resistance profile. *Pseudomonas aeruginosa*/genetic study. *Acinetobacter* spp./antimicrobial resistance profile. *Acinetobacter* spp./genetic study. Antimicrobial resistance. Bacterial typing.

No Brasil, bem como em outras regiões do mundo, *Pseudomonas aeruginosa* e *Acinetobacter* spp. surgiram como importantes agentes de infecção nosocomial e são comumente envolvidos em surtos. O objetivo principal deste estudo foi descrever a relação genética de *P. aeruginosa* e *Acinetobacter* spp. isoladas de pacientes internados em hospital universitário público do noroeste do Paraná - Brasil e reportar o perfil de resistência dessas bactérias. Um total de 75 *P. aeruginosa* e 94 *Acinetobacter* spp. isolados foi fenotipicamente identificado e testado para a suscetibilidade aos antibióticos por metodologia automatizada. A polimixina B foi testada por difusão em disco para *P. aeruginosa*. Metalo-β-lactamase (MBL) foi detectada por disco-aproximação. Análise genotípica foi realizada por *enterobacterial repetitive intergenic consensus polymerase chain reaction* (ERIC-PCR). Aproximadamente 55% dos isolados de *P. aeruginosa* e 92% de *Acinetobacte*r spp. isolados foram multirresistentes, mas nenhum foi produtor de MBL. Os resultados de ERIC-PCR revelaram pequenos grupamentos de *Acinetobacter* spp. resistentes aos carbapenêmicos, provavelmente pela produção de carbapenemases do tipo OXA. Além disso, alta diversidade genética entre os isolados de *P. aeruginosa* e *Acinetobacter* spp. foi observada, sugerindo que a transmissão cruzada destas espécies bacterianas não é muito frequente em nosso hospital.

**Unitermos**: *Pseudomonas aeruginosa/*perfil de resistência. *Pseudomonas aeruginosa*/estudo genético. *Acinetobacter* spp./perfil de resistência. *Acinetobacter* spp./estudo genético. Resistência antimicrobiana. Tipagem bacteriana.

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## INTRODUCTION

*Pseudomonas aeruginosa* and *Acinetobacter* spp. have been the cause of nosocomial outbreaks worldwide (Cortes *et al*., 2009; Hosoglu *et al*., 2011), including in Brazil (Brito *et al*., 2003; Prates *et al*., 2011), where multidrug resistance is common among these isolates (Rossi, 2011). Carbapenems often represent the only effective treatment. However, carbapenem-resistant isolates have frequently been detected, leading clinicians to resume the use of older classes of antibiotics, such as polymyxins (Giamarellou, 2010). The production of carbapenemase enzymes has been an important mechanism of resistance in these bacteria. Metallo-β-lactamases (MBLs), enzymes able to hydrolyze all β-lactam antibiotics with the exception of monobactams, are particularly common in *P. aeruginosa*, and the genes that encode them are carried by highly mobile elements that play an important role in hospital environments (Cornaglia, Giamarellou, Rossolini, 2011).

Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) has shown good applicability in typing *A. baumannii* and *P. aeruginosa* (Presterl *et al*., 1997; Syrmis *et al*., 2004; Kidd *et al*., 2011). Although some studies have used ERIC-PCR to epidemiologically study *P. aeruginosa* and *Acinetobacter* spp. nosocomial isolates in Brazil (Costa *et al*., 2006; Saalfeld *et al*., 2009; Stehling, Leite, Silveira, 2010; Ferreira *et al*., 2011; Viana *et al*., 2011), the specific epidemiology of these microorganisms remains unknown.

The main objective of the present study was to describe the genetic relationship among *P. aeruginosa* and *Acinetobacter* spp. clinical isolates using ERIC-PCR and report their resistance profiles against antimicrobial agents that are routinely used in a public university hospital in northwestern Paraná, Brazil.

## MATERIAL AND METHODS

Seventy-five *P. aeruginosa* and 94 *Acinetobacter* spp. isolates from various clinical specimens from patients admitted to a public hospital in northwestern Paraná, Brazil, between January 2007 and July 2009 were studied. Only one isolate from each patient was selected and stored at -80 ºC in the Laboratory of Medical Bacteriology, Department of Clinical Analysis and Biomedicine, State University of Maringá. No personal data were retrieved from the patients, so privacy could be assured and legal transgressions concerning human research could be avoided (Resolution 196/96 Brazil National Health Council, Health Ministry). The present study was approved by the Regulatory Commission of Academic Activities and Voluntary Services of the studied hospital.

Phenotypic identification and antimicrobial susceptibility testing (AST) were performed using an AUTOSCAN-4 automated system (Siemens Microscan, Deerfield, IL, USA), with the exception of polymyxin B for *P. aeruginosa*, which was tested by disk diffusion. The data were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI; 2007-2009).

Ceftazidime-resistant *P. aeruginosa* and *Acinetobacter* spp. isolates were evaluated for the presence of MBL using the disk-approximation test, 2-mercaptopropionic acid (2-MPA; Acros, Bridgewater, NJ, USA), and ethylenediaminetetraacetic acid (EDTA; Invitrogen, Carlsbad, CA, USA; Arakawa *et al*. 2000). IMP-1-producing *Acinetobacter baumannii* (A-3227) and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively.

Genomic DNA of *P. aeruginosa* and *Acinetobacter* spp. isolates was extracted from overnight bacterial growth on Mueller-Hinton agar (Difco, Becton Dickinson, Sparks, MD, USA; Swanenburg *et al*., 1998). The PCRs were performed using primers ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') as described by Szczuka and Kaznowski (2004). The gels were stained with ethidium bromide, and the spectral band analysis on agarose gels was performed using BioNumerics software (version 4.45, Applied Maths, Sint-Martens-Latem, Belgium). The Dendrogram was constructed using the Dice coefficient, and the phylogenetic distance was determined using the Unweighted Pair Group Method with Arithmetic Mean algorithm (Sneath and Sokal, 1973). Isolates with ≥ 95% similarity were considered closely related.

## RESULTS

The studied institution is a small-size public hospital with three intensive care units (ICUs): adult, pediatric, and neonatal. *P. aeruginosa* and *Acinetobacter* spp. were isolated more frequently in male patients (62.7% and 59.6%, respectively), especially in patients older than 60 years (54.7% and 39.4%, respectively) and in ICUs (41.4% and 71.3%, respectively). *P. aeruginosa* isolates were detected at a higher frequency in urine samples, whereas *Acinetobacter* spp. isolates were mainly recovered from tracheal aspirate.

The resistance rate in *P. aeruginosa* isolates was less than 30% for most of the β-lactam antibiotics, and 54.6% were multiresistant (i.e., resistant to three or more antimicrobial classes; Magiorakos *et al*., 2012). All of the isolates were polymyxin B-susceptible. For *Acinetobacter*

spp. isolates, the β-lactam resistance rate was greater than 90% to cephalosporins and greater than 50% to the tested carbapenems (Table I). Multiresistance was observed in 91.5%. None of the *P. aeruginosa* ($n$ = 39) and *Acinetobacter* spp. ($n$ = 88) isolates with total or intermediate resistance to ceftazidime were positive for MBL.

**TABLE I -** Resistance profiles of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates from inpatients in a public hospital in northwestern Paraná, Brazil

| Antimicrobial agent | Resistant isolates (%) | |
|---|---|---|
| | *P. aeruginosa* ($n$ = 75) | *Acinetobacter* spp. ($n$ = 94) |
| Aztreonam | 13 (29.3)[a] | NT |
| Amikacin | 30 (40.0) | 53 (56.4) |
| Ampicillin/Sulbactam | NT | 21 (27.6)[b] |
| Cefepime | 22 (29.3) | 87 (92.5) |
| Ceftazidime | 21 (28.0) | 85 (90.4) |
| Ciprofloxacin | 37 (49.3) | 88 (93.6) |
| Gentamicin | 33 (44.0) | 70 (74.5) |
| Imipenem | 19 (25.4) | 52 (54.8) |
| Meropenem | 19 (25.4) | 53 (56.4) |
| Piperacillin/Tazobactam | 10 (13.3) | NT |
| Polymyxin B | 0.0 (0.0) | NT |
| Trimethoprim/ Sulfamethoxazole | NT | 88 (93.6) |

NT, not tested; [a]Tested isolates = 46; [b]Tested isolates = 76.

The ERIC-PCR applied to *P. aeruginosa* and *Acinetobacter* spp. clinical isolates showed a banding pattern with sizes that rang, from approximately 120 pb to 1200 pb and 120 pb and 1900 pb, respectively.

Considering a Dice correlation coefficient ≥ 0.95, 72 ERIC-PCR patterns were obtained in 75 *P. aeruginosa* clinical isolates. Seventy isolates (93.3%) were orphans, and the remaining five (6.7%) were included in two clusters comprising two (cluster A) and three (cluster B) isolates (Figure 1).

Two isolates that belonged to cluster A showed identical antimicrobial resistance profiles and were isolated from patients in different clinical units within a time interval of approximately 2 months. Isolates that belonged to cluster B, two with 100% similarity, had equal resistance profiles and were isolated from patients in an adult ICU within a time interval of only 4 days. The third isolate that belonged to cluster B, despite having the same resistance profile, was isolated 13 days later in a patient admitted to the pediatric unit (Table II).

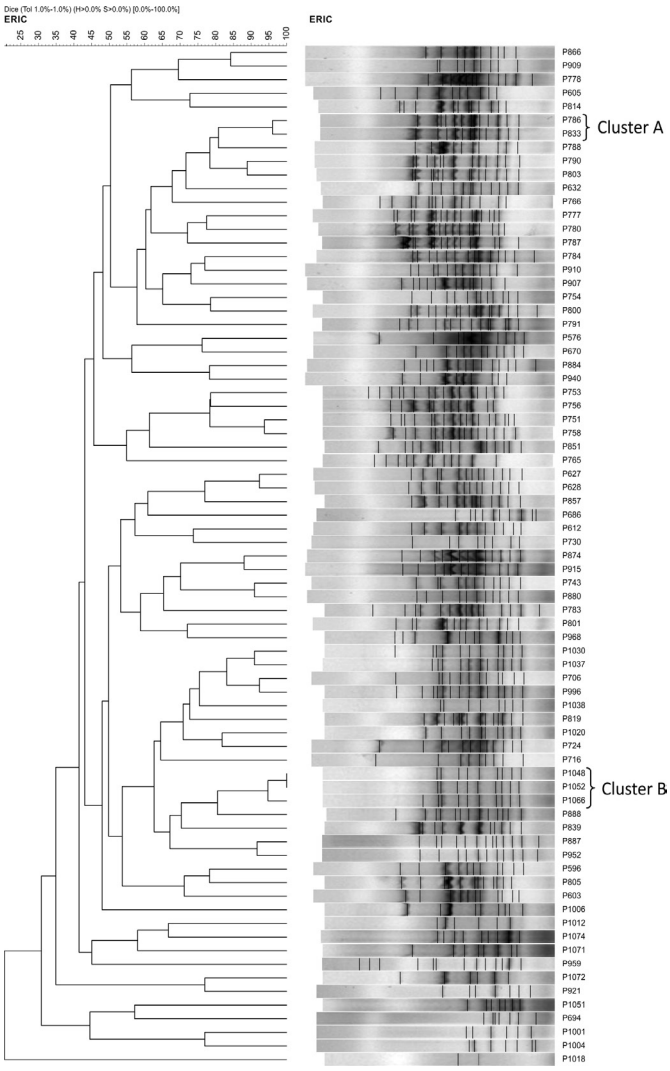


**FIGURE 1.** Dendrogram and DNA-banding profiles that represent the genetic relationship among *Pseudomonas aeruginosa* isolated from clinical specimens of inpatients in a public hospital in northwestern Paraná, Brazil.

A total of 70 *Acinetobacter* spp. isolates (74.5%) showed orphan ERIC-PCR patterns, and the remaining 24 (25.5 %) were included in 10 clusters (C-L) comprising two isolates each, with the exception of clusters D and H with three isolates each (Figure 2).

The several small clusters found in *Acinetobacter* spp. were generally detected in the same unit or units with displacement of patients, such as the adult ICU and medical clinic. Exceptions were observed in clusters D and J, which were composed of isolates from the adult and pediatric ICUs. *Acinetobacter* clusters included isolates with similar resistance profiles, with some differences related to aminoglycosides and carbapenems. Notably, 60% of the small clusters were carbapenems-resistant *Acinetobacter* spp. (Table II).

**TABLE II -** Demographics and phenotypic and genotypic features of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates with similarity ≥ 95%

| Cluster | Microorganism (isolate) | Clinical Specimens | Date of Isolation | Hospital Ward | Antimicrobial Resistance Pattern |
|---|---|---|---|---|---|
| A | *P. aeruginosa* (786) | Urine | 4 Mar 2008 | Pediatrics | Susceptible to all tested antimicrobials |
| A | *P. aeruginosa* (833) | Tracheal aspirate | 12 May 2008 | Adult ICU | Susceptible to all tested antimicrobials |
| B | *P. aeruginosa* (1048) | Tracheal aspirate | 12 Jun 2009 | Adult ICU | Cip; Azt; Cef; Cfz |
| B | *P. aeruginosa* (1052) | Blood | 8 Jun 2009 | Adult ICU | Cip; Azt; Cef; Cfz |
| B | *P. aeruginosa* (1066) | Urine | 25 Jun 2009 | Medical clinic | Cip; Azt; Cef; Cfz |
| C | *Acinetobacter* spp. (885) | Urine | 13 Jul 2008 | Medical clinic | Amp/Sul; Cip; Sut; Cef; Cfz |
| C | *Acinetobacter* spp. (893) | Tracheal aspirate | 14 Aug 2008 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami |
| D | *Acinetobacter* spp. (1033) | Tracheal aspirate | 14 May 2009 | Pediatric ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| D | *Acinetobacter s*pp. (1067) | Tracheal aspirate | 24 Jun 2009 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| D | *Acinetobacter* spp. (1043) | Tracheal aspirate | 25 May 2009 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| E | *Acinetobacter* spp. (1002) | Urine | 4 Mar 2009 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| E | *Acinetobacter* spp. (1024) | Blood | 23 Mar 2009 | Medical clinic | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| F | *Acinetobacter* spp. (900) | Tracheal aspirate | 20 Aug 2008 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami |
| F | *Acinetobacter* spp. (918) | Tracheal aspirate | 24 Sep 2008 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami |
| G | *Acinetobacter* spp. (573) | Urine | 17 Feb 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| G | *Acinetobacter* spp. (585) | Blood | 15 Mar 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Imp; Mer |
| H | *Acinetobacter* spp. (559) | Surgical wound | 29 Jan 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| H | *Acinetobacter* spp. (564) | Tracheal aspirate | 29 Jan 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| H | *Acinetobacter* spp.(548) | Tracheal aspirate | 3 Jan 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| I | *Acinetobacter* spp. (611) | Tracheal aspirate | 17 Apr 2007 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| I | *Acinetobacter* spp. (613) | Tracheal aspirate | 26 Apr 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Imp; Mer |
| J | *Acinetobacter* spp. (554) | Tracheal aspirate | 16 Jan 2007 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami |
| J | *Acinetobacter* spp. (557) | Tracheal aspirate | 4 Feb 2007 | Pediatric ICU | Cip; Sut; Cef; Cfz; Gen; Ami |
| K | *Acinetobacter* spp. (566) | Tracheal aspirate | 22 Jan 2007 | Medical clinic | Cip; Sut; Cef; Cfz; Ami |
| K | *Acinetobacter* spp. (568) | Tracheal aspirate | 15 Feb 2007 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami |
| L | *Acinetobacter* spp. (610) | Tracheal aspirate | 17 Apr 2007 | Adult ICU | Amp/Sul; Cip; Sut; Cef; Cfz; Gen; Ami; Imp; Mer |
| L | *Acinetobacter* spp. (617) | Tracheal aspirate | 11 May 2007 | Adult ICU | Cip; Sut; Cef; Cfz; Gen; Ami |

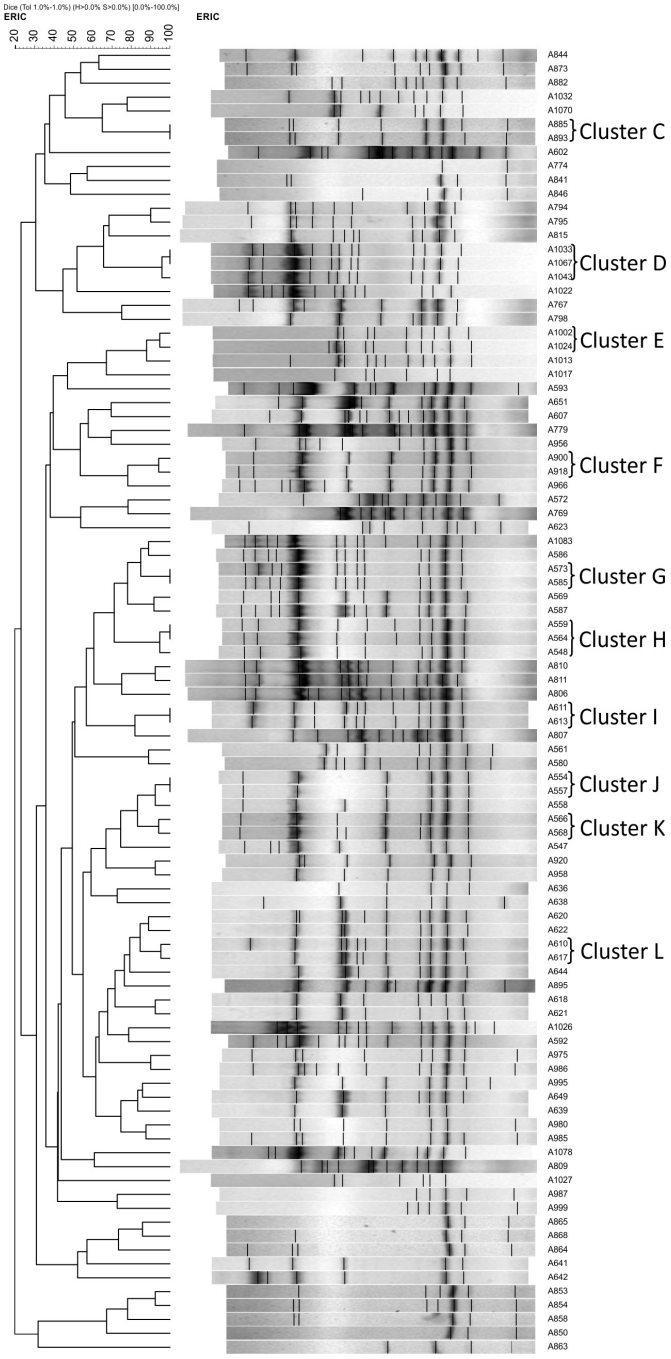


**FIGURE 2.** Dendrogram and DNA-banding profiles that represent the genetic relationship among *Acinetobacter* spp. isolated from clinical specimens of inpatients in a public hospital in northwestern Paraná, Brazil.

## DISCUSSION

In the present study, the ERIC-PCR assessment and antimicrobial resistance profiles of *P. aeruginosa* and *Acinetobacter* spp. isolates from inpatients in a small-size teaching hospital in northwestern Paraná showed a high percentage of multiresistance and genetic diversity. Worrying rates of ceftazidime and carbapenems resistance were observed in *P. aeruginosa* isolates. However, they were smaller than those observed in isolates from other university hospitals in Brazil, where resistance to these antimicrobials was near or greater than 50% (Zavascki, Cruz, Goldani, 2004; Rocha *et al*., 2008).

In recent years, MBL production has represented an important mechanism of resistance to most β-lactam antibiotics, including carbapenems, especially in *P. aeruginosa* (Kunz, Brook, 2010). However, despite a resistance rate of just over 25% detected for carbapenems, none of the *P. aeruginosa* isolates tested were positive for MBL, demonstrating the probable participation of other resistance mechanisms. The isolation rates of MBL-producing *P. aeruginosa* have widely varied among Brazilian hospitals, with incidences ranging from 3.1% (Wirth *et al*., 2009) to 35% (Gonçalves *et al.*, 2009). According to Rossi (2011), São Paulo metallo-β-lactamase (SPM) is the most prevalent MBL in Brazilian isolates. For *Acinetobacter* spp. isolated in the present study, the observed resistance rates of > 90% for third-generation cephalosporins and nearly 55% for carbapenems may be responsible for the difficulty treating these cases, which has been reported in other studies (Towner, 2009; Kunz, Brook, 2010). MBL was not detected in isolates from this genus, which corroborates other studies that found a low contribution of this enzyme to the increasing rates of resistance in *Acinetobacter* spp. (Mostachio *et al*., 2009; Kunz, Brook, 2010). OXA-type β-lactamases have shown an important role in carbapenem-resistant *Acinetobacter* spp. (Poirel, Naas, Nordmann, 2010). In *Acinetobacter* spp. isolates from Brazilian hospitals, carbapenem resistance is mostly related to β-lactamase OXA-23 (Gales *et al*., 2012). However, a recent study found a high prevalence of OXA-143 in these bacterial genera (Mostachio *et al*., 2012). Therefore, the small clusters of carbapenem-resistant *Acinetobacter* spp. isolated in this study may have been OXA-type carbapenemase producers.

Although clonal spread is commonly found in hospitals worldwide (Scott and Pitt, 2004; Cortes *et al.*, 2009), including Brazil (Stheling, Leite, Silveira, 2010), the present study detected high genetic diversity, especially among *P. aeruginosa* isolates. Only two (2.7%) of the 75 isolates showed identical ERIC-PCR patterns (100% similarity), and five (6.7%) had ≥ 95% similarity. However, according to the phenotypic features and demographic conditions, the cross-spread of small clusters of *P. aeruginosa* and *Acinetobacter* spp., including carbapenem-resistant isolates, among patients in the hospital, was evident. Importantly, however, the findings of Saalfeld *et al.* (2009) should be

considered, in which high genetic similarity was found among clinical and environmental *Acinetobacter* spp. isolates in an adult ICU in the same hospital from January to July 2008. These authors suggested that an endemic situation existed by observing the genotypic similarity of isolates using a Dice correlation coefficient > 90%.

## CONCLUSION

Using ERIC-PCR, the present study found high genetic diversity among *P. aeruginosa* and *Acinetobacter* spp. isolates, suggesting that cross-contamination is not very frequent in the studied hospital. Because of the known applicability of ERIC-PCR, its good discriminatory power and reproducibility allowed an understanding of the epidemiology of these bacteria in the studied hospital environment.

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