Diversity of metallo-β-lactamase-encoding genes found in distinct species of Acinetobacter isolated from the Brazilian Amazon Region

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BACKGROUND The multidrug resistance (MDR) phenotype is frequently observed in Acinetobacter baumannii, the most clinically relevant pathogenic species of its genus; recently, other species belonging to the A. calcoaceticus-A. baumannii complex have emerged as important MDR nosocomial pathogens.

OBJECTIVES The present study aimed to verify the occurrence of metallo-β-lactamase genes among distinct Acinetobacter species in a hospital located in the Brazilian Amazon Region.

METHODS Antimicrobial susceptibility profiles were determined by broth microdilution. The genetic relationships among these isolates were assessed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Pyrosequencing reads of plasmids carrying the blaNDM-1 gene were generated using the Ion Torrent™ platform sequencing.

FINDINGS A total of six isolates carried blaNDM-1: A. baumannii (n = 2), A. nosocomialis (n = 3), and A. pittii (n = 1); three carried blaIMP-1: A. baumannii, A. nosocomialis, and A. bereziniae. Resistance to colistin was observed for an NDM-1-producing Acinetobacter baumannii isolate. Diverse PFGE patterns and sequence types were found among A. nosocomialis and A. baumannii isolates. The blaNDM-1 sequence was inserted in a Tn125 transposon, while the blaIMP-1 was found as a gene cassette of the class 1 integron In86.

MAIN CONCLUSIONS To the best of our knowledge, this is the first report describing the dissemination of blaNDM-1 among distinct Acinetobacter species recovered from the same hospital in South America.

Key words: metallo-β-lactamase - non-baumannii Acinetobacter species - polymyxin resistance - nosocomial infection - ICU - Tn125

The World Health Organization (WHO) has recognised carbapenem-resistant Acinetobacter baumannii as a critical priority pathogen for antimicrobial development and research.10 The multidrug resistance (MDR) phenotype is frequently observed in A. baumannii, which is the most clinically relevant species of its genus,10 and recently, other species belonging to the A. calcoaceticus-A. baumannii complex have emerged as important MDR nosocomial pathogens.3,4,5,6

According to the most recent report from the Brazilian Health Surveillance Agency (ANVISA), Acinetobacter spp. were ranked as the fourth most frequent pathogen (n = 2,129; 12.6%) causing catheter-related bloodstream infections (CR-BSI) in Brazilian intensive care units (ICUs).7 Eighty-five percent of Acinetobacter spp. reported to ANVISA was resistant to carbapenems. Therefore, polymyxins have constituted the first therapeutic option for the treatment of serious carbapenem-resistant Acinetobacter spp. infections. In Brazil, carbapenem resistance among A. baumannii clinical isolates is mainly associated with the production of the class D carbapenemase (CHDL) variants OXA-23, OXA-72, and OXA-143 at a lower frequency.3,8,9 The spread of high-risk clones of producers of OXA-23 or OXA-72 explain the high carbapenem resistance rates observed among Acinetobacter spp. recovered from adults diagnosed with CR-BSI in Brazilian ICUs.7

In contrast, carbapenem resistance due to the production of metallo-β-lactamas (MβLs) has rarely been reported in A. baumannii as well as in non-baumannii species.4,10 IMP-type MβLs have only been reported in Acinetobacter spp. isolated from hospitals located in the state of São Paulo,4,10 south-eastern Brazil, while NDM-1-producing isolates have been sporadically described in hospitals located in the three different states of the Southern region.3,6,11

Herein, we report the spread of NDM-1 and IMP-1-producing Acinetobacter spp. causing complicated nosocomial infections at a Brazilian tertiary hospital located in the Brazilian Amazon Region (Northern Brazilian Region), and the emergence of polymyxin resistance in this group of pathogens.

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Bacterial isolates and phenotypic detection of MβL production - A total of 478 non-duplicated Acinetobacter spp. clinical isolates were obtained between January 2012 and June 2016 at a tertiary teaching hospital with 408 beds located in the city of Belém, state of Pará, Brazil. The isolates were sent to Evandro Chagas Institute (IEC) for further characterisation. Among those isolates, nine (1.9%) showed reduced susceptibility to carbapenems and were positive by imipenem/imipenem+EDTA (0.5 M) double disk synergy test. Subsequently, the MβL phenotype was confirmed using imipenem/imipenem+EDTA Etest® strips (bioMérieux, Solna, Sweden) according to the manufacturer’s instructions.

Species identification and antimicrobial susceptibility testing - Species identification was performed by partial sequencing of the rpoB gene, as previously described. Minimum inhibitory concentrations (MICs) of ampicillin/sublactam, ceftazidime, cefotaxime, imipenem, meropenem, ciprofloxacin, amikacin, gentamicin, tobramycin, minocycline, tigecycline, polymyxin B, and colistin (Sigma-Aldrich, St. Louis, USA) were determined by cation-adjusted broth microdilution (Oxoid, Basingstoke, UK) following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The susceptibility results were interpreted according to the current EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints).

Detection of carbapenemase-encoding genes - Polymerase chain reaction (PCR) followed by DNA sequencing was performed for the detection of acquired CHDL (bla<sub>NDM</sub>-like, bla<sub>OXA-2</sub>-like, bla<sub>OXA-24</sub>-like, bla<sub>OXA-38</sub>-like, bla<sub>OXA-141</sub>-like) and MβL-encoding genes (bla<sub>NDM</sub>-like, bla<sub>IMP</sub>-like, bla<sub>VIM</sub>-like, bla<sub>SIP</sub>-like and bla<sub>PDM</sub>-like) using specific primers, as previously described. Amplicons were purified using the QIAquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) according to the manufacturer’s instructions, and sequencing reactions were prepared using the BigDye Terminator Cycle Sequencing Kit (Qiagen, Courtaboeuf, France) according to the manufacturer’s instructions, and sequencing reactions were prepared using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and the ABI 3500 Genetic Analyser (Applied Biosystems, Perkin Elmer, USA). The nucleotide sequences and the derived protein sequences were analysed using the Lasergene Software Package (DNASTAR, Madison, USA), and then compared with those deposited in the GenBank database.

Characterisation of plasmids and genetic context of MβL-encoding genes - Genomic and plasmid DNA were extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer’s recommendations. The genetic structures surrounding the MβL-encoding genes were evaluated by PCR mapping followed by DNA sequencing using specific primers based on previously reported sequences. The plasmids carrying bla<sub>NDM</sub> were digested with endonuclease S1 and resolved by pulsed-field gel electrophoresis (PFGE), followed by DNA-DNA hybridisation assay that was assessed by Southern blotting using a Hybond™-N nylon transfer membrane (GE Healthcare, Little Chalfont, UK). DIG-labelling of a bla<sub>NDM</sub>-1-specific probe and signal detection was performed using the DIG DNA Labelling and Detection Kit (Roche Diagnostics GmbH, Penzberg, Germany).

Genotyping by PFGE and multilocus sequence typing (MLST) - The clonal relationships of MβL-producing Acinetobacter spp. isolates belonging to the same species were investigated by PFGE using the CHEF DR II system (Bio-Rad, Hercules, California) and Apal restriction enzyme (New England BioLabs, MA, USA), as previously described. MLST was performed for those isolates belonging to the A. calcoaceticus-A. baumannii complex by double-stranded DNA sequencing of internal regions of seven housekeeping genes (cpxA, fesA, gltA, pyrG, recA, rplB, and rpoB) according to the Institute Pasteur scheme. Determination of the sequence type (ST) was performed through the A. baumannii MLST website (http://pubmlst.org/abaumannii/). The relationships between novel and existing STs were surveyed using the eBURST program (http://eburst.mlst.net/).

Genomic DNA sequencing, assembly, and plasmid sequence analysis - Genomic DNA from representatives of each Acinetobacter species carrying bla<sub>NDM</sub> was extracted and purified using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) according to the manufacturer’s protocol. Single-end pyrosequencing reads of plasmids were generated using the Ion Torrent™ Personal Genome Machine™ (PGM) platform sequencing (Thermo Fisher Scientific, Carlsbad, CA). Raw sequence reads were trimmed and assembled de novo using plasmidSPAdes version 3.9.0 (http://bioinf.spbau.ru/spades) and MIRA v.1.0.4 software, respectively, followed by gap filling by manual assembly. Each draft plasmid was annotated by Rapid Annotations using Subsystems Technology (RAST) (http://rast.nmpdr.org) and further manually curated by Geneious v.9.1.6 (Biomatters Limited, Auckland, New Zealand) using Basic Local Alignment Search Tool (BLAST) against the non-redundant NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Nucleotide sequencing accession numbers - The complete nucleotide sequences of plasmids pIEC383, pIEC37710, and pIEC38057 were submitted to GenBank under accession numbers MK053932, MK053933, and MK053934, respectively.

Ethical approval - Ethical approval for the study was obtained from Research Ethics Committee from Evandro Chagas Institute (process number: 655.019/CAAE: 24147014.8.0000.0019).

RESULTS AND DISCUSSION

Among the nine carbapenem-resistant Acinetobacter spp. isolates phenotypically identified as MβL producers, six harboured bla<sub>NDM</sub> (n = 6) and three carried bla<sub>IMP</sub> (n = 3) genes (Table). No acquired CHDL-encoding gene was found in these isolates. The NDM-1-producing Acinetobacter spp. isolates were identified as A. baumannii (n = 2), A. nosocomialis (n = 3), and A. pittii (n = 1), while the three IMP-1-producing isolates were identified as A. baumannii, A. nosocomialis, and A. bereziniae. MβL-producing Acinetobacter spp. isolates were mostly recovered

MATERIALS AND METHODS

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between March and August 2014 (n = 6). Most patients infected by these pathogens (n = 6; 66.7%) were hospitalised at paediatric or neonatal ICUs, as shown in Table; these results concur with those reported by previous studies. Acinetobacter spp. have previously been reported to be the 6th and 10th most frequent pathogens in Brazilian paediatric (n = 135; 6.5%) and neonatal ICUs (n = 199; 3.1%) in 2016, showing carbapenem resistance rates of 21% and 44%, respectively.\(^7\) While primary BSI was documented in five of six patients infected with NDM-1-producing Acinetobacter spp., the IMP-1-producing isolates were recovered from surgical wounds, peritoneal fluid, and urine. Only two patients infected with NDM-1-producing isolates died during hospitalisation (Table).

Expectedly, MβL-producing Acinetobacter spp. isolates showed high MIC values to all β-lactams tested (Table). Contrastingly, minocycline and tigecycline were the most active antimicrobial agents against the isolates in vitro, with MIC\(_{90}\) values of ≤ 0.25 μg/mL and 0.5 μg/mL, respectively. Such results corroborated previous data that also showed good in vitro activity of minocycline against IMP-1- and IMP-10-producing Acinetobacter spp. isolates in Brazil.\(^{4,10}\) Despite its potential activity, the minocycline formulation for intravenous administration (Minocin\(^\text{®}\)) is not yet available in Brazil. The NDM-1-producing Acinetobacter spp. isolates were also susceptible to ciprofloxacin and aminoglycosides. In contrast, most of the IMP-1-producing Acinetobacter spp. isolates were resistant to these drugs (Table). Interestingly, an IMP-1-producing A. nosocomialis isolate (IEC195) showed a high resistance rate to colistin (MIC, > 64 μg/mL). To the best of our knowledge, this is the first report of a colistin-resistant A. nosocomialis strain carrying bla\(_{\text{NDM-1}}\). Notably, a worrisome decrease in the susceptibility to polymyxins was observed among all NDM-1-producing A. nosocomialis and IMP-1-producing A. bereziniae isolates (MICs varying from 1-2 μg/mL). According to a study conducted by Vila-Farrés and colleagues, who evaluated induced colistin-resistant A. nosocomialis mutants, the loss of LPS due to mutations in the lpxD gene was found to be the main mechanism associated with this resistance phenotype in this species.\(^{11}\) In another study, Wang and colleagues reported high resistance rates to colistin (21.4%; n = 58/271) among A. nosocomialis isolates recovered from BSI in a hospital located in Taiwan during an 11-year period.\(^{12}\) The authors also reported that the colistin resistance could not be transferred by a conjugation assay, indicating a chromosomal mechanism that corroborated those findings previously reported by Vila-Farrés and colleagues.\(^{13}\) In addition, all 58 colistin-resistant A. nosocomialis isolates evaluated by Wang and colleagues were susceptible to ciprofloxacin,\(^{14}\) which was also observed in the colistin-resistant IEC195 strain (MIC 0.5 μg/mL) in the present study.

PFGE analysis showed that the three MβL-producing A. baumannii isolates were not clonally related. Contrastingly, the isolates IEC383 and IEC429 carrying the blal\(_{\text{NDM-1}}\) and blal\(_{\text{IMP-1}}\) genes, respectively, belonged to the same ST (ST464/CC464), while the IEC304 were included in ST216/CC216, as shown in Table. To date, only one NDM-1-producing A. baumannii isolate has been report-

| Species identification, clinical data, genetic similarity, carbapenemase content, and antimicrobial susceptibility profile of nine carbapenem-resistant Acinetobacter spp. isolates | MIC (μg/mL) |
|---|---|
| Isolates | Date of isolation | Clinical specimen | Species | Age | Underlying disease | Isolate | Hospital unit | Species identification | Genetic similarity | Carbapenemase content | Antimicrobial susceptibility profile |
| IEC304 | 02/18/2014 | 66 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC304 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC336 | 02/28/2013 | 7 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC336 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC403 | 07/12/2016 | 44 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC403 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC383 | 02/12/2014 | 66 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC383 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC429 | 03/28/2014 | 66 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC429 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC38057 | 10/12/2016 | 3 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC38057 | ICU | Acinetobacter baumannii | ST216/CC216 | IMP-10 | nalidixic acid 0.5, tobramycin 16, tigecycline 8, minocycline 0.25, fusidic acid 0.5, rifampicin 0.25, vancomycin 1, amikacin 16, aztreonam 1, imipenem 0.25, ticarcillin-clavulanate 16, ceftazidime 0.25, ciprofloxacin 0.125, gentamicin 0.125, netilmicin 0.0625, polymyxin B 0.125, polymyxin E 2 |
| IEC195 | 03/28/2014 | 16 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC195 | ICU | A. nosocomialis | | | |
| IEC338 | 06/26/2014 | 66 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC338 | ICU | A. nosocomialis | | | |
| IEC343 | 08/28/2013 | 128 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC343 | ICU | A. nosocomialis | | | |
| IEC38058 | 06/28/2014 | 2 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC38058 | ICU | A. nosocomialis | | | |
| IEC383 | 06/28/2014 | 2 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC383 | ICU | A. nosocomialis | | | |
| IEC338 | 06/28/2014 | 2 y | Nosocomial pneumonia | Blood | Nosocomial pneumonia | IEC338 | ICU | A. nosocomialis | | | |

MIN: minimal inhibitory concentration; IMP: imipenem; MIN: minocycline; NI: not informed; SAM: ampicillin/sulbactam; TGC: tigecycline; TOB: tobramycin.
ed in Brazil, and it belonged to ST25/CC25. The other two NDM-1-producing A. baumannii strains reported in the continent were both from Colombia, and one of them also belonged to ST464. Interestingly, none of the STs associated with NDM-1-producing A. baumannii strains described in South America (Fig. 1) belonged to the major clonal complexes CC1, CC15, or CC79, which was responsible for the spread of OXA-23, and more recently, of OXA-72 in A. baumannii isolated from this geographic region. Furthermore, the four MβL-producing A. nosocomialis isolates showed distinct PFGE patterns and belonged to CC410 (ST71 and ST1075), CC782 (ST433), and to the singleton ST279 (Table). Rojas and colleagues reported an NDM-1-producing A. nosocomialis isolate belonging to ST322 harbouring the same plasmid as that found in an A. baumannii strain recovered in Colombia (Fig. 1).

According to S1 nuclease-PFGE/hybridisation analysis, blaNDM-1 was located in a ~45-kb plasmid in all six Acinetobacter spp. isolates. Previous reports described similar plasmids (~45-55 kb) carrying blaNDM-1 in distinct Acinetobacter species in South America (Fig. 1). This MβL-encoding gene was also reported in a ~100-kb plasmid found in A. baumannii, and in the chromosomes of A. pittii strains recovered from other Brazilian medical centres.

Initially, the sequencing of a 5,251-bp fragment flanking blaNDM-1 revealed the presence of the aminoglycoside-modifying enzyme (AME)-encoding gene aphA6, followed by ISAba125 upstream of blaNDM-1, which was followed downstream by bleMBL (bleomycin resistance) and AtrpF (phosphoribosylanthranilate isomerase) genes in all NDM-1-producing Acinetobacter spp. isolates. This conserved genetic structure (ISAba125–bleMBL–ΔtrpF) suggests that blaNDM-1 was inserted in the composite transposon Tn125, which has been commonly associated with the mobilisation of this MβL-encoding gene. To date, Tn125 has been described among distinct NDM-1-producing Acinetobacter species (Fig. 1) recovered from Argentina, Brazil, Colombia, and Paraguay.

Notably, plasmid sequence analysis revealed that blaNDM-1 was carried by a 47,283-bp plasmid (pIEC383; Fig. 2A) in the A. baumannii IEC383 strain and in a 41,085-bp plasmid (pIEC38057; Fig. 2B) in the A. nosocomialis IEC38057 strain that displayed 86% identity at the nucleotide level, corroborating the results obtained by S1 nuclease-PFGE/hybridisation assays. The pIEC383 and pIEC38057 plasmids displayed 95% and 100% identity with pAbNDM-1 and pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwoffii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively. In addition, both plasmids harboured genes encoding a type IV secretion system that could be related to plasmid conjugation and for a Z toxin of unknown function (Fig. 2A-B). For A. pittii IEC37710, only a fragment (13,363 bp, Fig. 2C) of the plasmid pIEC37710 was obtained, displaying 99% identity with pNDM-BJ01 isolated from A. baumannii (JN377410) and A. lwofii (JQ060896) recovered in China, respectively.
strains (Fig. 1A,C) were identical to those commonly described among distinct Acinetobacter species carrying bla_{NDM-1} recovered in Argentina and Colombia (Fig. 1). However, the comparison between the Tn125 structures found in A. pittii IEC37710 and the other Brazilian A. pittii strain carrying bla_{NDM-1} in the chromosome was not possible (Fig. 1), since the Tn125 had not been fully sequenced. Moreover, the Tn125 found in A. nosocomialis pIEC38057 showed an unusual structure composed of a truncated cutA with a resolvase-encoding gene tnpR downstream of tat, and the complete absence of groES-groEL-insE, as well as the right-side copy of ISAbal25 (Fig. 1). This deletion of 6,198 bp explains the differences in the plasmid sizes observed between pIEC383 (47,283 bp; Fig. 2A) and pIEC38057 (41,085 bp; Fig. 2B). These results demonstrate a high variability of the Tn125 genetic backbones among distinct Acinetobacter species in South America (Fig. 1), contrasting with the high homology (99-100%) verified among plasmids carrying bla_{NDM-1} worldwide.15,16,17

The analysis of the genetic context of bla_{IMP-1} showed that it was carried by the class 1 integron In86 in all Acinetobacter spp. isolates. The cassette arrangement structure of In86 is composed of bla_{IMP-1} at the first position, followed by two AME-encoding genes (aacA31 and aadA1e), as previously described.4 The In86 has been described among distinct Acinetobacter species, and in other gram-negative bacilli recovered from hospitals in the southeastern Brazil. Interestingly, the distance between the cities of Belém and São Paulo, where IMP-like-producing Acinetobacter spp. strains had been geographically restricted, is 2,465 km (1,532 miles).

In conclusion, we reported the spread of NDM-1 and IMP-1 among distinct Acinetobacter species in Brazil. The acquisition of MBL-encoding genes by non-baumannii Acinetobacter species is of great concern, drastically limiting the therapeutic options for infections caused by such pathogens. In addition, the production of class B carbapenemases seems to be the main mechanism of carbapenem resistance among non-baumannii Acinetobacter species in the Brazilian Amazon Region, as well as in other South American countries. The successful dissemination of bla_{NDM-1} among different hosts seems to be mainly associated with the plasmid-mediated composite transposon Tn125 in this geographic region. Therefore, attention should be paid to control the spread of these emerging multidrug-resistant pathogens, particularly among patients hospitalised at ICUs.

AUTHORS’ CONTRIBUTION
DB, RC and ACG conceived and supervised the project; RS provided the Acinetobacter isolates for the study; DB, RC, APS, CSN, RRB, PSL and JMM carried out the experimental procedures; YC and IM collected patient clinical data; DB, RC, APS, CSN and ACG drafted the manuscript. All authors read and approved the final manuscript. Competing interests: ACG recently received research funding and/or consultation fees from Bayer, Eurofarma, Pfizer, and MSD. Other authors have nothing to declare. This study was not financially supported by any Diagnostic/Pharmaceutical company.

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