

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

Danielle Brasiense^{1/+}, Rodrigo Cayô², Ana Paula Streling³, Carolina S Nodari³, Rafael R Barata⁴, Poliana S Lemos⁴, Janaina M Massafra⁴, Yan Correa¹, Igor Magalhães¹, Ana C Gales³, Roberta Sodré⁵

¹Instituto Evandro Chagas, Seção de Bacteriologia e Micologia, Ananindeua, PA, Brasil

²Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Departamento de Ciências Biológicas, Setor de Biologia Molecular, Microbiologia e Imunologia, Diadema, SP, Brasil

³Universidade Federal de São Paulo, Departamento de Medicina, Escola Paulista de Medicina, Laboratório Alerta, Disciplina de Infectologia, São Paulo, SP, Brasil

⁴Instituto Evandro Chagas, Centro de Inovação Tecnológica, Ananindeua, PA, Brasil

⁵Hospital Fundação Santa Casa de Misericórdia do Pará, Belém, PA, Brasil

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 *bla*_{NDM-1} gene were generated using the Ion Torrent™ platform sequencing.

FINDINGS A total of six isolates carried *bla*_{NDM-1}: *A. baumannii* (n = 2), *A. nosocomialis* (n = 3), and *A. pittii* (n = 1); three carried *bla*_{IMP-1}: *A. baumannii*, *A. nosocomialis*, and *A. bereziniae*. Resistance to colistin was observed for an NDM-1-producing *A. nosocomialis* isolate. Diverse PFGE patterns and sequence types were found among *A. nosocomialis* and *A. baumannii* isolates. The *bla*_{NDM-1} sequence was inserted in a Tn125 transposon, while the *bla*_{IMP-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 *bla*_{NDM-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.⁽¹⁾ The multidrug resistance (MDR) phenotype is frequently observed in *A. baumannii*, which is the most clinically relevant species of its genus,⁽²⁾ 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 carbapen-

ems. 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 -lactamases (M β LS) has rarely been reported in *A. baumannii* as well as in non-*baumannii* species.^(4,10,11) IMP-type M β LS 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.^(5,6,11)

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.

doi: 10.1590/0074-02760190020

Financial support: This work was supported by Evandro Chagas Institute, CAPES [grants to APS and CSN (DS-CAPES)], CNPq (grant to ACG - Process nº 305535/2014-5).

+ Corresponding-author: daniellemurici@iec.gov.br

https://orcid.org/0000-0003-2772-639X

Received 17 January 2019

Accepted 29 April 2019



MATERIALS AND METHODS

Bacterial isolates and phenotypic detection of M β L 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 M β L 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.⁽¹²⁾ Minimum inhibitory concentrations (MICs) of ampicillin/sulbactam, 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*_{OXA-23}-like, *bla*_{OXA-24/40}-like, *bla*_{OXA-58}-like, *bla*_{OXA-143}-like) and M β L-encoding genes (*bla*_{NDM}-like, *bla*_{IMP}-like, *bla*_{VIM}-like, *bla*_{SIM}-like and *bla*_{SPV}-like) using specific primers, as previously described.^(8,9,10) 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 (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 M β L-encoding genes - Genomic and plasmid DNA were extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) based on the manufacturer's recommendations. The genetic structures surrounding the M β L-encoding genes were evaluated by PCR mapping followed by DNA sequencing using specific primers based on previously reported sequences.^(4,10) The plasmids carrying *bla*_{NDM-1} 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).⁽¹⁰⁾ DIG-labelling of a *bla*_{NDM-1}-spe-

cific 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 M β L-producing *Acinetobacter* spp. isolates belonging to the same species were investigated by PFGE using the CHEF DR II system (Bio-Rad, Hercules, California) and ApaI restriction enzyme (New England BioLabs, MA, USA), as previously described.⁽⁹⁾ 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 (*cpn60*, *fusA*, *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*_{NDM-1} 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 M β L producers, six harboured *bla*_{NDM-1} (n = 6) and three carried *bla*_{IMP-1} (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*. M β L-producing *Acinetobacter* spp. isolates were mostly recovered

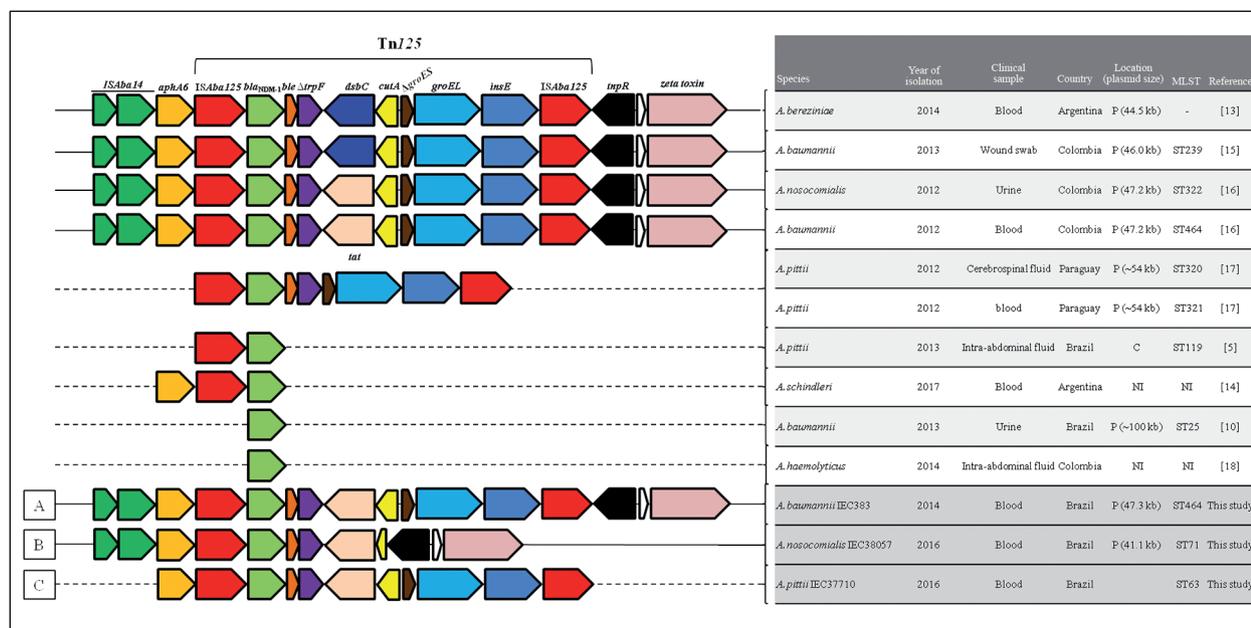


Fig. 1: comparative analysis of Tn125 backbone described in all current NDM-1-producing *Acinetobacter* species reported in South America with those found in the present study (A-C). Dashed lines represent the inner or surrounding regions of Tn125 that were not sequenced. White arrows represent open reading frames (ORFs) that encoded hypothetical proteins. Genes and their transcription orientation were indicated by arrows (not to scale). P: plasmid; C: chromosome; NI: not informed.

ed in Brazil, and it belonged to ST25/CC25.⁽¹¹⁾ 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.^(15,16) 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.⁽³⁾ Furthermore, the four M β L-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).⁽¹⁶⁾ Furthermore, the NDM-1-producing *A. pittii* isolate (IEC37710) reported in the present study was included in ST63/CC63 (Table), differing from the two previous studies that described this M β L in *A. pittii* strains recovered from Brazil (ST119)⁽⁵⁾ and Paraguay (ST320 and ST321).⁽¹⁷⁾

According to S1 nuclease-PFGE/hybridisation analysis, *bla*_{NDM-1} was located in a ~45-kb plasmid in all six *Acinetobacter* spp. isolates. Previous reports described similar plasmids (~45-55 kb) carrying *bla*_{NDM-1} in distinct *Acinetobacter* species in South America (Fig. 1). This M β L-encoding gene was also reported in a ~100-kb plasmid found in *A. baumannii*,⁽¹¹⁾ and in the chromosome of *A. pittii* strains recovered from other Brazilian medical centres.⁽⁵⁾

Initially, the sequencing of a 5,251-bp fragment flanking *bla*_{NDM-1} revealed the presence of the aminoglycoside-modifying enzyme (AME)-encoding gene

aphA6, followed by *ISAbal125* upstream of *bla*_{NDM-1}, which was followed downstream by *ble*_{MBL} (bleomycin resistance) and *AtrpF* (phosphoribosylanthranilate isomerase) genes in all NDM-1-producing *Acinetobacter* spp. isolates. This conserved genetic structure (*ISAbal125-bla*_{NDM-1}-*ble-AtrpF*) suggests that *bla*_{NDM-1} was inserted in the composite transposon Tn125, which has been commonly associated with the mobilisation of this M β L-encoding gene.^(5,15,16,17,18,19,20) To date, Tn125 has been described among distinct NDM-1-producing *Acinetobacter* species (Fig. 1) recovered from Argentina,^(18,19,20) Brazil,^(5,20) Colombia,^(15,16,20) and Paraguay.^(17,20)

Notably, plasmid sequence analysis revealed that *bla*_{NDM-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-GJ01 (30,293 bp) isolated from *A. towneri* (KT965092). Interestingly, the three NDM-1-producing *Acinetobacter* species showed distinct Tn125 backbones (Fig. 1). The Tn125 structures found in *A. baumannii* IEC383 and in *A. pittii* IEC37710

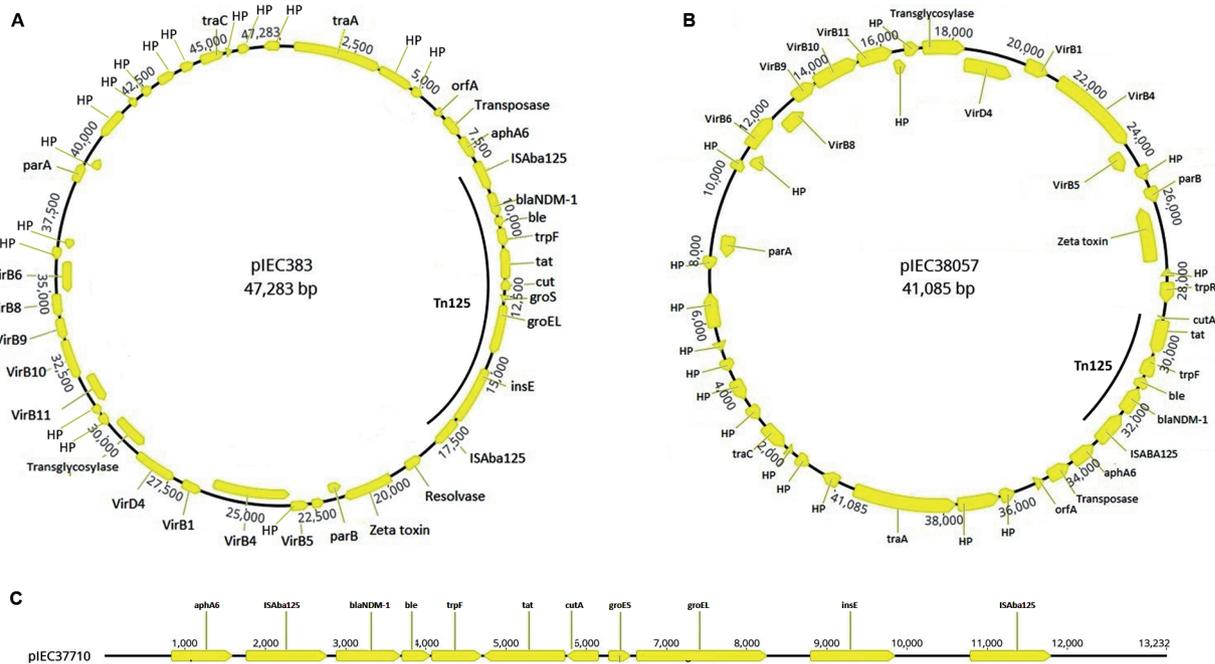


Fig. 2: (A) circular map of plasmid pIEC383 recovered from *Acinetobacter baumannii* IEC383. The yellow arrows represent the coding sequences and the direction of transcription. HP: hypothetical protein. The Tn125 region can be observed. (B) Circular map of plasmid pIEC38057 recovered from *A. nosocomialis* IEC38057. The legend of Fig. 2B was same as that of Fig. 2A. (C) Partial sequence of plasmid pIEC37710 recovered from *A. pittii* IEC37710.

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).^(15,16,18,20) 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.⁽⁵⁾ 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.⁽⁴⁾ Interestingly, the distance between the cities of Belém and São Paulo, where IMP-like-producing *Acinetobacter* spp. strains had been geographically restricted,^(4,10) 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 M β L-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.

REFERENCES

- Guidelines for the prevention and control of carbapenem-resistant enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. Geneva: World Health Organization. 2017.

2. Wong D, Nielsen TB, Bonomo RA, Pantapalangkoor P, Luna B, Spellberg B. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clin Microbiol Rev*. 2017; 30(1): 409-47.
3. Vasconcelos AT, Barth AL, Zavascki AP, Gales AC, Levin AS, Lucarevski BR, et al. The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. *Diagn Microbiol Infect Dis*. 2015; 83(4): 382-5.
4. Cayô R, Streling AP, Nodari CS, Matos AP, de Paula Luz A, Dijkshoorn L, et al. Occurrence of IMP-1 in non-*baumannii* *Acinetobacter* clinical isolates from Brazil. *J Med Microbiol*. 2018; 67(5): 628-30.
5. Pagano M, Poirer L, Martins AF, Rozales FP, Zavascki AP, Barth AL, et al. Emergence of NDM-1-producing *Acinetobacter pittii* in Brazil. *Int J Antimicrob Agents*. 2015; 45(4): 444-5.
6. Chagas TP, Carvalho-Assef AP, Aires CAM, Bertocini R, Asensi MD. Detection of an NDM-1-producing *Acinetobacter bereziniae* strain in Brazil. *J Glob Antimicrob Resist*. 2015; 3(2): 147-8.
7. ANVISA (Brazilian Health Surveillance Agency). Boletim segurança do paciente e qualidade em serviços de saúde nº16: avaliação dos indicadores nacionais das infecções relacionadas à assistência à saúde (IRAS) e resistência microbiana do ano de 2016. Accessed on 08 September 2018. Available from: <https://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoes/item/boletim-seguranca-do-paciente-e-qualidade-em-servicos-de-saude-n-16-avaliacao-dos-indicadores-nacionais-das-infecoes-relacionadas-a-assistencia-a-saude-iras-e-resistencia-microbiana-do-ano-de-2016>.
8. Rodrigues-Costa F, Cayô R, Matos AP, Girardello R, Martins WMBS, Carrara-Marroni FE, et al. Temporal evolution of *Acinetobacter baumannii* ST107 clone: conversion of bla(OXA-143) into bla(OXA-231) coupled with mobilization of IS*AbaI* upstream *occA**BI*. *Res Microbiol*. 2019; 170(1): 53-9.
9. Cardoso JP, Cayô R, Girardello R, Gales AC. Diversity of mechanisms conferring resistance to β -lactams among OXA-23-producing *Acinetobacter baumannii* clones. *Diagn Microbiol Infect Dis*. 2016; 85(1): 90-7.
10. Cayô R, Rodrigues-Costa F, Matos AP, Carvalhaes CG, Jové T, Gales AC. Identification of a new integron harboring bla(IMP-10) in carbapenem-resistant *Acinetobacter baumannii* clinical isolates. *Antimicrob Agents Chemother*. 2015; 59(6): 3687-9.
11. Pillonetto M, Arend L, Vespero EC, Pelisson M, Chagas TP, Carvalho-Assef AP, et al. First report of NDM-1-producing *Acinetobacter baumannii* sequence type 25 in Brazil. *Antimicrob Agents Chemother*. 2014; 58(12): 7592-4.
12. La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol*. 2006; 44(3): 827-32.
13. Vila-Farrés X, Ferrer-Navarro M, Callarisa AE, Martí S, Espinal P, Gupta S, et al. Loss of LPS is involved in the virulence and resistance to colistin of colistin-resistant *Acinetobacter nosocomialis* mutants selected *in vitro*. *J Antimicrob Chemother*. 2015; 70(11): 2981-6.
14. Wang YC, Lee YT, Yang YS, Chen CT, Chiu CH, Yin T, et al. Risk factors and outcome for colistin-resistant *Acinetobacter nosocomialis* bacteraemia in patients without previous colistin exposure. *Clin Microbiol Infect*. 2015; 21(8): 758-64.
15. Marquez-Ortiz RA, Haggerty L, Olarte N, Duarte C, Garza-Ramos U, Silva-Sanchez J, et al. Genomic epidemiology of NDM-1-encoding plasmids in Latin American clinical isolates reveals insights into the evolution of multidrug resistance. *Genome Biol Evol*. 2017; 9(6): 1725-41.
16. Rojas LJ, Wright MS, De La Cadena E, Motoa G, Hujer KM, Villegas MV, et al. Initial assessment of the molecular epidemiology of bla_{NDM-1} in Colombia. *Antimicrob Agents Chemother*. 2016; 60(7): 4346-50.
17. Pasteran F, Mora MM, Albornoz E, Faccione D, Franco R, Ortellado J, et al. Emergence of genetically unrelated NDM-1-producing *Acinetobacter pittii* strains in Paraguay. *J Antimicrob Chemother*. 2014; 69(9): 2575-8.
18. Brovedan M, Marchiaro PM, Morán-Barrio J, Cameranesi M, Cera G, Rinaudo M, et al. Complete sequence of a bla(NDM-1)-harboring plasmid in an *Acinetobacter bereziniae* clinical strain isolated in Argentina. *Antimicrob Agents Chemother*. 2015; 59(10): 6667-9.
19. Montaña S, Palombarani S, Carulla M, Kunst A, Rodriguez CH, Nastro M, et al. First case of bacteraemia due to *Acinetobacter schindleri* harbouring bla(NDM-1) in an immunocompromised patient. *New Microbes New Infect*. 2017; 21: 28-30.
20. Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther*. 2017; 15(3): 277-97.