Acinetobacter baumannii infections in Amazon Region driven by extensively drug resistant international clones, 2016-2018

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BACKGROUND Acinetobacter baumannii is a leading cause of nosocomial infections. This species is characterised by the presence of pandemic lineages (International Clones) that present a broad antimicrobial resistance profile.

OBJECTIVE To perform the molecular epidemiology of carbapenem-resistant A. baumannii from a clinical setting in the Amazon Basin, and to characterise their antimicrobial resistance determinants.

METHODS The genetic relationship of carbapenem-resistant A. baumannii were assessed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Class A, B and D β-lactamase genes were screened by polymerase chain reaction (PCR) and sequencing. The antimicrobial susceptibility profile was obtained by Disc-diffusion method and minimum inhibitory concentration (MIC) determination.

FINDINGS All carbapenem-resistant A. baumannii strains belonged to three international clones, IC-1, IC-5 and IC-6, the latter recently reported by the first time in Brazil. The major determinant of carbapenem resistance in IC-1 and IC-5 strains was blaOXA-23, associated with ISAbal and ISAba3, respectively, while IC-6 harboured the blaOXA-72.

CONCLUSIONS The A. baumannii epidemiology in Brazilian Amazon Region was unknown. It was demonstrated that A. baumannii XDR international clones were responsible for nosocomial infections in Boa Vista during 2016-2018, revealing that the epidemiological scenario of A. baumannii infections in Amazon Region resembles those from the cosmopolitan regions worldwide.

Key words: international clone - extensively drug resistance - Acinetobacter baumannii - Amazon Region - blaOXA-23

Acinetobacter baumannii has emerged in recent years as a leading cause of nosocomial infections associated with a longer hospital stay and higher mortality, representing a public health problem of major concern worldwide. A. baumannii presents the long-term ability to survive on inanimate surfaces, and this persistence seems to contribute to its person-to-person transmission, intra- and inter-hospital outbreak spread, and national and international clonal dissemination. Additionally, this species is characterised by remarkable capabilities for the acquisition of antibiotic resistance genes (ARGs).

High-risk pandemic lineages, named international clones (ICs), presenting high capacity to persist in clinical environments and a broad antimicrobial resistance profile have been associated with outbreaks in several cosmopolitan regions around the world. Carbapenem resistance is a major therapeutic concern in Acinetobacter and it is usually mediated by carbapenem-hydrolysing class D β-lactamase (CHDL) from OXA Family, such as blaOXA-23-like, blaOXA-58-like, blaOXA-24-like, blaOXA-143 and blaOXA-235. In Brazil, the carbapenem resistance rates are around 80.7%-86% as a consequence of blaOXA-23 dissemination by some pandemic lineages, such as those from CC15, CC79 and CC1 (international clone IC-1). The blaOXA-23 is frequently found downstream the ISAbal sequence, which accounts for its mobilisation and supports its overexpression due to the presence of a strong promoter.

The epidemiology of clinical A. baumannii and its antibiotic resistance determinants are concentrated in densely populated cosmopolitan cities from the South and Southeast Brazilian regions. Considering that Brazil is a country with continental dimensions and with contrasting demographical features, it is crucial to gain insights about the epidemiological scenario and the dynamics of carbapenem resistance in other geographical regions outside these cosmopolitan sites.

This study aimed to determine the molecular epidemiology of carbapenem-resistant A. baumannii strains from a clinical setting of the North region, and to characterise their carbapenem resistance determinants.

MATERIALS AND METHODS

Clinical data, bacterial strains and antimicrobial susceptibility test - The General Hospital of Roraima (GHR), placed in Boa Vista, is a 281-bed tertiary healthcare medical unit, the largest in Roraima, which includes general wards, two intensive care units (ICUs) with 10 beds each, surgical centre and emergency.
From October, 2016 to May, 2018, 101 *A. baumannii* isolates were recovered from nosocomial infections cases of non-repetitive inpatients. From 101 isolates, 27 were resistant to carbapenem and these strains were characterised as described below (Table). Species identification was performed with the automated VITEK2, and confirmed by sequencing the 16S rRNA and the bla*_{OXA,SI}*, genes.

The antibiotic susceptibility profile was determined by disc-diffusion method according to clinical and laboratory standards institute (CLSI) guidelines,(14) for the following antibiotics: gentamicin, amikacin, tobramycin, imipenem, meropenem, doripenem, ciprofloxacin, ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, trimethoprim/sulphamethoxazole, tetracycline and minocycline. The minimum inhibitory concentration (MIC) of polymyxin B was assessed by the broth microdilution with antibiotic concentrations ranged from 0.1 μg/mL to 64 μg/mL. The current definition criteria for classifying *A. baumannii* antimicrobial resistance was applied.(15) The carbapenemase production was assessed by the modified Hodge Test.(16)

**Genotyping by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST)** - The clonal relationship among the carbapenem-resistant *A. baumannii* strains was establish by the in situ lysis technique, in agarose blocks as described previously and digested with 30U Apal restriction enzyme. PFGE banding

| Strains | Isolation date | PFGE | MLST (IC) | Ward | Clinical specimen | Carbapenemase activity | ISAb*{a} -bla*_{OXA} |
|---------|----------------|------|-----------|------|------------------|------------------------|--------------------|
| AB4332  | Oct/16/16      | B    | ST78 (IC-6) | ICU  | Tracheal secretion | +                      | OXA-72             |
| AB4353  | Oct/21/16      | A    | ST79 (IC-5) | ICU  | Catheter tip      | +                      | ISAb*{a}3-OXA-23   |
| AB5262  | Dec/21/16      | B    | ST78 (IC-6) | ICU  | Catheter tip      | +                      | OXA-72             |
| AB5375  | Dec/29/16      | B    | ST78 (IC-6) | Traumatology | Tracheal secretion | +                      | OXA-72             |
| AB49    | Jan/03/17      | C    | ST1 (IC-1)  | ICU  | Catheter tip      | +                      | ISAb*{a}1-OXA-23   |
| AB77    | Jan/05/17      | A    | ST79 (IC-5) | others hospital wards | Wound secretion | +                      | ISAb*{a}3-OXA-23   |
| AB715   | Feb/12/17      | A    | ST79 (IC-5) | Emergency | Bronchial aspirate | +                      | ISAb*{a}3-OXA-23   |
| AB1077  | Mar/08/17      | A    | ST79 (IC-5) | ICU  | Tracheal secretion | +                      | ISAb*{a}3-OXA-23   |
| AB1113  | Mar/08/17      | A    | ST79 (IC-5) | ICU  | Cerebrospinal fluid | +                      | ISAb*{a}3-OXA-23   |
| AB283   | Apr/25/17      | C    | ST1 (IC-1)  | ICU  | Tracheal secretion | +                      | ISAb*{a}1-OXA-23   |
| AB08    | Aug/31/17      | B    | ST78 (IC-6) | ICU  | Blood             | +                      | OXA-72             |
| AB65    | Sep/26/17      | C    | ST1 (IC-1)  | others hospital wards | Bone tissue | +                      | ISAb*{a}1-OXA-23   |
| AB81    | Oct/02/17      | A1   | ST79 (IC-5) | ICU  | Tracheal secretion | +                      | ISAb*{a}3-OXA-23   |
| AB04-R5 | Jan/01/18      | A1   | ST79 (IC-5) | ICU  | Blood             | +                      | ISAb*{a}3-OXA-23   |
| AB07-R5 | Jan/14/18      | C    | ST1 (IC-1)  | others hospital wards | Blood | +                      | ISAb*{a}1-OXA-23   |
| AB28-R5 | Jan/17/18      | A    | ST79 (IC-5) | ICU  | Tracheal secretion | +                      | ISAb*{a}3-OXA-23   |
| AB37-R5 | Jan/19/18      | A    | ST79 (IC-5) | ICU  | Tracheal secretion | +                      | ISAb*{a}3-OXA-23   |
| AB40-R5 | Jan/19/18      | C    | ST1 (IC-1)  | ICU  | Tracheal secretion | +                      | ISAb*{a}1-OXA-23   |
| AB39-R5 | Jan/21/18      | C    | ST1 (IC-1)  | ICU  | Tracheal secretion | +                      | ISAb*{a}1-OXA-23   |
| AB51-R5 | Jan/26/18      | A    | ST79 (IC-5) | ICU  | Tracheal secretion | +                      | ISAb*{a}3-OXA-23   |
| AB07-R6 | Apr/23/18      | C    | ST1 (IC-1)  | others hospital wards | Wound secretion | +                      | ISAb*{a}1-OXA-23   |
| AB04-R6 | Apr/25/18      | A    | ST79 (IC-5) | ICU  | Catheter tip      | +                      | ISAb*{a}3-OXA-23   |
| AB06-R6 | Apr/26/18      | C    | ST1 (IC-1)  | ICU  | Hepatic abscess   | +                      | ISAb*{a}1-OXA-23   |
| AB01-R6 | Apr/29/18      | B    | ST78 (IC-6) | ICU  | Catheter tip      | +                      | OXA-72             |
| AB05-R6 | Apr/29/18      | A    | ST79 (IC-5) | others hospital wards | Catheter tip | +                      | ISAb*{a}3-OXA-23   |
| AB02-R6 | May/03/18      | C    | ST1 (IC-1)  | ICU  | Tracheal secretion | +                      | ISAb*{a}1-OXA-23   |
| AB03-R6 | May/15/18      | B    | ST78 (IC-6) | ICU  | Tracheal secretion | +                      | OXA-72             |

ICU: intensive care unit; MLST (IC): multilocus sequence typing (international clone); PFGE: pulsed-field gel electrophoresis.

**TABLE**
Epidemiological, phenotypic and genotypic features of the XDR *Acinetobacter baumannii* international clones found in General Hospital of Roraima, Boa Vista
patterns were analysed and compared visually. Isolates were considered to be clonal when the macrorestriction DNA patterns differed by fewer than three bands.\(^{(17)}\)

The MLST was performed using the Oxford an Pasteur schemes (https://pubmlst.org/abaumannii/\(^{3}\)) available in the A. baumannii MLST website (https://pubmlst.org/abaumannii/\(^{4,18}\)). Clonal complexes (CCs) were considered when sequence types (STs) shared five or more identical alleles taking into account the seven genes that are considered in the MLST schemes.\(^{(3)}\)

Detection of carbapenem resistance genes by polymerase chain reaction (PCR) and sequencing - The presence of genes encoding class A (\(bla_{KPC}, bla_{VIM}, bla_{NDM}\)), class B (\(bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{QP}, bla_{SIM}, \) and \(bla_{NDM}\)), and class D (\(bla_{OXA-23}, \) and \(bla_{OXA-48}\)) \(\beta\)-lactamases with carbapenemase activity was detected by PCR and sequencing in the carbapenem-resistant isolates as previously described.\(^{(19,20,21,22,23,24)}\)

The presence of the IS\(Aba1\) upstream the \(bla_{OXA}\) genes was also investigated.\(^{(25)}\)

**RESULTS AND DISCUSSION**

The phenotypic analysis revealed that all carbapenem-resistant strains (\(n = 27\)) presented the XDR phenotype, since they were susceptible only to polymyxin B, minocycline and tetracycline (Table). All strains were positive for the Modified Hodge Test, indicating carbapenemase production.

PFGE and MLST analyses demonstrated the concomitant occurrence of three XDR lineages in HGR from 2016 to 2018. The ST\(1^{(9,10)}, ST109^{(5)}\) (Clone C; \(n = 9\)), ST\(79^{(5)}\) (Clone A; \(n = 12\)) and ST\(79^{(5)}\) (Clone B; \(n = 6\)) corresponded to the high-risk pandemic International Clone I (IC-1), International Clone V (IC-5) and International Clone VI (IC-6), respectively (Table).\(^{(20,26,27,28,29)}\) Previous studies had already reported the dissemination and the high prevalence of multidrug resistant A. baumannii from CC1 (ST\(1^{(9,10,10)}\)) to CC77 (ST\(79^{(5)}, IC-5\) in Brazil.\(^{(9,10,10)}\) Here, it was demonstrated that CC1 and CC77 are prevalent in a clinical setting from the Amazon Region (Table). Interestingly, considering Brazil, the IC-6 seems to be restricted, so far, to this clinical setting in the Amazon Region,\(^{(29)}\) although it has been involved with outbreaks worldwide since 2006.\(^{(5,30)}\) These findings stress the spatial temporal persistence and dissemination potential of these three pandemic lineages, since they also occurred in the Brazilian Amazon Region, at least, during 2016-2018.

The \(bla_{OXA-23}\) in association with IS\(Aba1\) sequences, is one of the most widespread CHDL among A. baumannii in Brazil, and it has been disseminated in the country by the high-risk pandemic lineages from CC1 (IC-1), CC77 (IC-5) and CC15. However, most of these studies focused on clinical settings placed in the Southeast and South regions of Brazil.\(^{(2,9,10,11,26)}\) Similarly, we verified that \(bla_{OXA-23}\) was also the most prevalent carbapenemase gene among IC-1 and IC-5 XDR A. baumannii from Boa Vista (Table), and that it was found downstream IS\(Aba1\) and IS\(Aba3\), explaining the observed carbapenem resistance in IC-1 and IC-5 strains, respectively. Instead, the IC-6 strains carried the carbapenemase \(bla_{OXA-72}\) gene flanked by XerC/XerD binding sites.\(^{(29)}\) However, in spite of that, it was previously demonstrated that, even in the absence of IS\(Aba\) sequences, \(bla_{OXA-72}\) had contributed to the carbapenem resistance.\(^{(29,33)}\)

**In conclusion** - This study unraveled, by the first time, the epidemiological context of A. baumannii infections in a city from the Amazon Region. This scenario resembled that observed in cosmopolitan regions around the world, since it was verified that the nosocomial infections that occurred in Boa Vista from 2016 to 2018 was concomitantly caused by three XDR international clones, IC-1, IC-5 and IC-6, the latter only recently reported in Brazil (more precisely, in Boa Vista city). Such situation is probably due to the A. baumannii long-term ability to persist and survive in hospital environments, together with the person-to-person transmission and the global human mobility. Therefore, these findings provided a more complete picture concerning the importance of high-risk pandemic clones in the international dissemination of resistance, reinforcing the need of an epidemiological tracking of A. baumannii XDR strains and its associated carbapenemase coding genes even outside densely populated cosmopolitan regions.

**AUTHORS’ CONTRIBUTION**

RVC and FSF - Performed some assays; ELF - performed some assays wrote and discussed the paper; LR - collected the bacterial isolates; ACV - conceived and conducted the study, discussed and wrote the paper.

**REFERENCES**

1. Zarrilli R, Pournaras S, Giannouli M, Tsakris A. Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages. Int J Antimicrob Agents. 2013; 41: 11-19.
2. Rocha IV, Xavier DE, Almeida KRH, Oliveira SR, Leal NC. Multidrug-resistant Acinetobacter baumannii clones persist on hospital inanimate surfaces. Braz J Infect Dis. 2018; 22(5): 438-41.
3. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol. 2007; 5(12): 939-51.
4. Diackourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One. 2010; 5(4): e10034.
5. Karah N, Sundsfjord A, Towner K, Samuelsen Ø. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of Acinetobacter baumannii. Drug Resist Updat. 2012; 15(4): 237-47.
6. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006; 12(9): 826-36.
7. Neves FC, Clemente WT, Linconop N, Paião ID, Neves PR, Romanelli RM, et al. Clinical and microbiological characteristics of OXA-23- and OXA-143-producing Acinetobacter baumannii in ICU patients at a teaching hospital, Brazil. Braz J Infect Dis. 2016; 20(6): 556-63.
8. Rossi F, Girardello R, Cury AP, Di Gioia TSR, Almeida Jr JN, Du-
9. Camargo CH, Tiba MR, Saes MR, Vasconcellos FM, Santos LF, Romero EC, et al. Population structure analysis of carbapenem-resistant Acinetobacter baumannii clinical isolates from Brazil reveals predominance of clonal complexes 1, 15, and 79. Antimicrob Agents Chemother. 2016; 60(4): 2545-7.

10. Chagas TP, Carvalho KR, Santos ICO, Carvalho-Assef AP, Asensi MD. Characterization of carbapenem-resistant Acinetobacter baumannii in Brazil (2008-2011): countrywide spread of OXA-23-producing clones (CC15 and CC79). Diagn Microbiol Infect Dis. 2014; 79(4): 468-72.

11. Pagano M, Nunes LS, Niada M, Barth AL, Martins AF. Comparative analysis of carbapenem-resistant Acinetobacter baumannii sequence types in southern Brazil: from the first outbreak (2007-2008) to the endemic period (2013-2014). Microb Drug Resist. 2019; 25(4): 538-42.

12. Medshkat Z, Amini Y, Sadeghian H, Salimizand H. ISAba1/blaOXA-23-like family is the predominant cause of carbapenem resistance in Acinetobacter baumannii and Acinetobacter nosocomialis in Iran. Infect Genet Evol. 2019; 71: 60-6.

13. Grosso F, Carvalho KR, Quinteira S, Ramos A, Carvalho-Assef AP, Asensi MD, et al. OXA-23-producing Acinetobacter baumanii: a new hotspot of diversity in Rio de Janeiro? J Antimicrob Chemother. 2011; 66(1): 62-5.

14. CLSI - Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing 22th informational supplement (M100-S27) 2017. Wayne: CLSI; 2017.

15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3): 268-81.

16. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 2001; 7(2): 88-91.

17. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995; 33: 2233-9.

18. Burtual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol. 2005; 43(9): 4382-90.

19. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward E, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006; 27: 351-3.