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 $bla_{\text{OXA-23}}$, associated with IS*Aba1* and IS*Aba3*, respectively, while IC-6 harboured the $bla_{\text{OXA-72}}$.

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 - $bla_{\text{OXA-23}}$

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.[1] *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.[2] Additionally, this species is characterised by remarkable capabilities for the acquisition of antibiotic resistance genes (ARGs).[3] 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.[4,5]

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 $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-143}}$ and $bla_{\text{OXA-235}}$.[1,6,7] In Brazil, the carbapenem resistance rates are around 80.7%[8] as a consequence of $bla_{\text{OXA-23}}$ dissemination by some pandemic lineages, such as those from CC15, CC79 and CC1 (international clone IC-1).[9,10,11] The $bla_{\text{OXA-23}}$ is frequently found downstream the IS*Aba1* sequence, which accounts for its mobilisation and supports its overexpression due to the presence of a strong promoter.[1,6,12]

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.[8,10,13] 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.

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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-51} 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 ApaI restriction enzyme. PFGE banding

TABLE

Epidemiological, phenotypic and genotypic features of the XDR *Acinetobacter baumannii* international clones found in General Hospital of Roraima, Boa Vista

Strains	Isolation date	PFGE	MLST (IC)	Ward	Clinical specimen	Carbapenemase activity	IS*Aba* -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	+	IS*Aba3*-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	+	IS*Aba1*-OXA-23
AB77	Jan/05/17	A	ST79 (IC-5)	others hospital wards	Wound secretion	+	IS*Aba3*-OXA-23
AB715	Feb/12/17	A	ST79 (IC-5)	Emergency	Bronchial aspirate	+	IS*Aba3*-OXA-23
AB1077	Mar/08/17	A	ST79 (IC-5)	ICU	Tracheal secretion	+	IS*Aba3*-OXA-23
AB1113	Mar/08/17	A	ST79 (IC-5)	ICU	Cerebrospinal fluid	+	IS*Aba3*-OXA-23
AB283	Apr/25/17	C	ST1 (IC-1)	ICU	Tracheal secretion	+	IS*Aba1*-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	+	IS*Aba1*-OXA-23
AB81	Oct/02/17	A1	ST79 (IC-5)	ICU	Tracheal secretion	+	IS*Aba3*-OXA-23
AB04-RR5	Jan/01/18	A1	ST79 (IC-5)	ICU	Blood	+	IS*Aba3*-OXA-23
AB07-RR5	Jan/14/18	C	ST1 (IC-1)	others hospital wards	Blood	+	IS*Aba1*-OXA-23
AB28-RR5	Jan/17/18	A	ST79 (IC-5)	ICU	Tracheal secretion	+	IS*Aba3*-OXA-23
AB37-RR5	Jan/19/18	A	ST79 (IC-5)	ICU	Tracheal secretion	+	IS*Aba3*-OXA-23
AB40-RR5	Jan/19/18	C	ST1 (IC-1)	ICU	Tracheal secretion	+	IS*Aba1*-OXA-23
AB39-RR5	Jan/21/18	C	ST1 (IC-1)	ICU	Tracheal secretion	+	IS*Aba1*-OXA-23
AB51-RR5	Jan/26/18	A	ST79 (IC-5)	ICU	Tracheal secretion	+	IS*Aba3*-OXA-23
AB07-RR6	Apr/23/18	C	ST1 (IC-1)	others hospital wards	Wound secretion	+	IS*Aba1*-OXA-23
AB04-RR6	Apr/25/18	A	ST79 (IC-5)	ICU	Catheter tip	+	IS*Aba3*-OXA-23
AB06-RR6	Apr/26/18	C	ST1 (IC-1)	ICU	Hepatic abscess	+	IS*Aba1*-OXA-23
AB01-RR6	Apr/29/18	B	ST78 (IC-6)	ICU	Catheter tip	+	OXA-72
AB05-RR6	Apr/29/18	A	ST79 (IC-5)	others hospital wards	Catheter tip	+	IS*Aba3*-OXA-23
AB02-RR6	May/03/18	C	ST1 (IC-1)	Emergency	Tracheal secretion	+	IS*Aba1*-OXA-23
AB03-RR6	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.

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/) 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.[5]

Detection of carbapenem resistance genes by polymerase chain reaction (PCR) and sequencing - The presence of genes encoding class A (bla_{KPC}, bla_{GES}, $bla_{B\text{-}KC}$), class B (bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM}, $bla_{SIM\text{-}1}$, and $bla_{NDM\text{-}1}$), and class D ($bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}143\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, $bla_{OXA\text{-}24/40\text{-}like}$, and $bla_{OXA\text{-}51\text{-}like}$) β-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 polymixin 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 $ST1^{PAS}$/$ST109^{OXF}$ (Clone C; n = 9), $ST79^{PAS}$/$ST758^{OXF}$ (Clone A; n = 12) and $ST78^{PAS}$/$ST944^{OXF}$ (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 (ST1/IC-1), CC15 (ST180) and CC79 (ST79/IC-5) in Brazil.[9,10,11] Here, it was demonstrated that CC1 and CC79 are also 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 $bla_{OXA\text{-}23}$, in association with IS*Aba* 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), CC79 (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 $bla_{OXA\text{-}23}$ 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 $bla_{OXA\text{-}72}$ 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, $bla_{OXA\text{-}72}$ had contributed to the carbapenem resistance.[29,31]

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.

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