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Emergence of extensively drug-resistant international clone IC-6 *Acinetobacter baumannii* carrying bla_{OXA-72} and $bla_{CTX-M-115}$ in the Brazilian Amazon region

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ABSTRACT

Objectives: The extensively drug-resistant (XDR) *Acinetobacter baumannii* international clone VI (IC-6) has been identified worldwide since 2006. This study reports the emergence of IC-6 in the Brazilian Amazon region and reveals the particular genomic features considering its mobilome and resistome.
Methods: A total of 32 carbapenem-resistant *A. baumannii* strains recovered from Boa Vista city (Roraima, Brazil) in 2016 were characterised by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The whole genome sequences of the Brazilian IC-6 strains were obtained. The mobilome and resistome were assessed by in silico analyses.
Results: PFGE and MLST demonstrated that the 32 *A. baumannii* strains belonged to four clones. One XDR clone corresponded to the high-risk pandemic IC-6 lineage from ST944^{Oxf}/78^{Pas}. The IC-6 resistome was composed of *aadA5*, *aac(3'')-IIa*, *aph(3')-Ia*, *armA*, *aadB*, *msrE*, bla_{TEM-1}, IS*15DIV*–$bla_{CTX-M-115}$–IS*15DIV*, bla_{OXA-90}, IS*Aba1*–$bla_{ADC-152}$, bla_{OXA-72}, *qacEΔ1* and *sul1*. Mobilome prediction revealed that bla_{OXA-72} was embedded in a 15.5-kb plasmid and that it was flanked by putative XerC/D-binding sites, possibly involved in bla_{OXA-72} mobilisation. Several resistance genes were in a 48-kb multidrug resistance genomic island inserted in the chromosome, which also harboured genes involved in host pathogenicity and adaptive traits. Interestingly, the Brazilian strains shared the bla_{OXA-72} and $bla_{CTX-M-115}$ with IC-6/ST944^{Oxf}/78^{Pas} recovered in a distinct spatiotemporal context, pointing to an epidemiological link among them.
Conclusion: This study highlights the importance of surveillance of XDR *A. baumannii* strains, even outside of densely populated cosmopolitan regions, to reveal the epidemiology of pandemic lineages, stressing their threat to public health.

1. Introduction

Acinetobacter baumannii pandemic lineages, named international clones, are spread across continents, causing outbreaks and presenting multidrug-resistant phenotypes. International clone VI (IC-6) belongs to the carbapenem-resistant *A. baumannii* ST944^{Oxf}/78^{Pas} [Oxford/Pasteur multilocus sequence typing (MLST) scheme] [1,2] and presents high biofilm-forming ability, increased resistance to desiccation and an enhanced capacity for host cell adhesion/invasion, favouring its diffusion and persistence [3]. Since 2006, IC-6 has been identified in European countries (Italy, Russia, Greece and Germany), Asia (Kuwait) and North and South America (the USA and French Guiana) [1,4–9].

In the context of surveillance of carbapenem-resistant *A. baumannii* infections in a hospital from the Brazilian Amazon region, we verified the occurrence of the pandemic IC-6/ST944^{Oxf}/78^{Pas} lineage and revealed particular genomic features considering its mobilome and resistome.

2. Materials and methods

A total of 32 nosocomial carbapenem-resistant *A. baumannii* strains were recovered from distinct inpatients, biological material and wards of the General Hospital of Roraima (Boa Vista, Roraima,

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Brazil) in 2016. Following identification using an automated VITEK® 2 system, the species was confirmed by sequencing of 16S rRNA and the bla_{OXA-51} gene, which is a genetic marker of this species. The strains were genotyped by pulsed-field gel electrophoresis (PFGE) and MLST following the Oxford and Pasteur schemes (https://pubmlst.org/abaumannii/) as described previously [1].

Antimicrobial susceptibility testing was performed by Etest (bioMérieux, Marcy-l'Étoile, France) on Mueller–Hinton agar for gentamicin, amikacin, tobramycin, imipenem, meropenem, doripenem, ciprofloxacin, ampicillin/sulbactam, piperacillin/tazobactam (TZP), ticarcillin/clavulanic acid, cefotaxime, ceftazidime, cefepime, trimethoprim/sulfamethoxazole (SXT), tetracycline and minocycline. The minimum inhibitory concentration (MIC) of polymyxin B was assessed by the broth microdilution method with antibiotic concentrations ranging from 0.1–64 μg/mL. Contemporary interpretive criteria from the Clinical and Laboratory Standards Institute (CLSI) were applied in all cases [10]. Carbapenemase production was verified by the modified Hodge test.

Whole-genome sequencing was performed on an Illumina HiSeq 2500 sequencer (Illumina Inc., San Diego, CA, USA) using a Nextera XT paired-end run with a 500-bp insert library at the High-Throughput Sequencing Platform of the Oswaldo Cruz Foundation (FIOCRUZ, Rio de Janeiro, Brazil).

In silico antimicrobial resistance gene (ARG) prediction and plasmid searches were performed using the Comprehensive Antibiotic Resistance Database (CARD), plasmidSPAdes and PlasmidFinder (https://cge.cbs.dtu.dk/services/). The plasmid topology (linear or circular) was verified in silico as previously described [11].

Genomic island prediction was assessed by IslandViewer web server (http://www.pathogenomics.sfu.ca/islandviewer/) and GIPSy software (http://www.bioinformatics.org/groups/?groupid=1180http://www.bioinformatics.org/groups/?groupid=1180), and insertion sequence (IS) elements were searched and classified according to the ISfinder database (http://www-is.biotoul.fr).

3. Results and discussion

The PFGE profile demonstrated that the 32 *A. baumannii* strains belonged to four clones: clone A ($n = 5$); clone B ($n = 15$); clone C ($n = 9$); and clone D ($n = 3$). Clones B, C and D were assigned as new STs by MLST. Interestingly, clone A belonged to ST944^{Oxf}/78^{Pas}, which corresponds to the high-risk pandemic IC-6 lineage.

According to the current resistance definition criteria [12], all five IC-6 strains presented an extensively drug-resistant (XDR) phenotype since the strains were susceptible only to polymyxin B and tetracyclines (tetracycline and minocycline) (Table 1). The remaining 27 strains were resistant or multidrug-resistant [12], with the highest resistance rates to meropenem, TZP and SXT.

To perform an in-depth characterisation of the Brazilian IC-6 strains, two strains were submitted to high-throughput sequencing. The genome sequences are found under the GenBank accession nos. **RJLV00000000** (AB4332) and **RJLW00000000** (AB5375).

Resistome mining of the Brazilian IC-6 genomes identified genes conferring resistance to aminoglycosides [*aadA5*, *aac(3″)-IIa*, *aph(3′)-Ia*, *armA* and *aadB*], macrolides (*msrE*), β-lactams and carbapenems (bla_{TEM-1}, $bla_{CTX-M-115}$, bla_{OXA-90}, $bla_{ADC-152}$ and bla_{OXA-72}), disinfectant compounds (*qacEΔ1*) and sulfonamides (*sul1*) (Table 2).

The bla_{OXA-90} gene, identified in the same chromosomal region both in strains AB4332 and AB5375, is a variant of the intrinsically-encoded bla_{OXA-51} family, which is a genetic marker of *A. baumannii* species. No IS*Aba* sequence was found associated with this gene.

Table 1
Susceptibility profile of five Brazilian *Acinetobacter baumannii* from the international clone VI (IC-6) lineage.

Antimicrobial agent	MIC (μg/mL)[a]
Gentamicin	≥256
Amikacin	≥256
Tobramycin	≥1024
Imipenem	≥32
Meropenem	≥32
Doripenem	16
Ciprofloxacin	≥32
Ampicillin/sulbactam	32
Piperacillin/tazobactam	≥256
Ticarcillin/clavulanic acid	≥256
Cefotaxime	≥256
Ceftazidime	≥256
Cefepime	≥256
Trimethoprim/sulfamethoxazole	≥32
Tetracycline	1
Minocycline	0.75
Polymyxin B	1

MIC, minimum inhibitory concentration.
[a] MICs were determined by Etest, except for polymyxin B which were determined by broth microdilution.

On the other hand, the $bla_{ADC-152}$ gene, also found in the chromosome, was preceded by IS*Aba1* (Table 2). Curiously, this allele presents amino acid substitutions (Asp220 and Gly320) that have been previously demonstrated to be involved in extension of the enzyme substrate specificity and acquisition of carbapenemase activity [13]. Therefore, the cephalosporin and carbapenem resistance observed in the Brazilian IC-6 strains can be also a consequence of the overexpression of $bla_{ADC-152}$ driven by IS*Aba1* [14].

The $bla_{CTX-M-115}$ gene was flanked by two IS*15DIV* elements (Table 2). However, it was not possible to determine whether the IS*15DIV*–$bla_{CTX-M-115}$–IS*15DIV* was located on a plasmid or on the chromosome since the assembled contigs were too small. IS*15DIV* belongs to the IS*6* family and has previously been identified flanking the bla_{NDM-16} gene carried by a plasmid in a clinical *Escherichia coli* strain [15] as well as upstream of $bla_{CTX-M-15}$ in *Klebsiella pneumoniae* clinical strains [16].

In silico analyses for plasmid prediction and topology determination revealed that a 15.5-kb contig corresponded to a circular plasmid carrying the conjugal transfer gene *traA* and the *virB6* gene as well as genes coding for the RelE/ParE toxin–antitoxin system. Moreover, this plasmid harboured bla_{OXA-72}, which was not associated with any IS element. Interestingly, it was previously demonstrated that bla_{OXA-72} contributes to carbapenem resistance even in the absence of IS*Aba* sequences [17–19].

Several studies have previously reported the presence of bla_{OXA-72} in different plasmids, STs, *Acinetobacter* spp. and countries, including Brazil [18–20]. These epidemiological data demonstrate the mobilisation potential of bla_{OXA-72}. The bla_{OXA-72} gene has been associated with binding sites homologous to those recognised by XerC/XerD recombinases [17,19,20], which could explain the heterogeneity of genetic contexts (different plasmids) in which bla_{OXA-72} has been found. In fact, the bla_{OXA-72} gene identified here was flanked by the same XerC/XerD putative binding sites previously reported in association with bla_{OXA-72} harboured by an 8.9-kb plasmid found in an IC-2 strain recovered from Italy in 2004 [17].

Besides the plasmid carrying bla_{OXA-72}, in silico mobilome mining revealed the presence of a 48-kb multidrug resistance genomic island (MDR GI) where the majority of the aforementioned ARGs were identified, including *aadA5*, *aac(3″)-IIa*, *aph(3′)-Ia*, *armA*, *aadB*, *msrE*, bla_{TEM-1}, *qacEΔ1* and *sul1*. Interestingly, several of these ARGs were flanked by ISs, which could account for their mobilisation and expression. This MDR GI, which was

Table 2
Epidemiological, phenotypic and genotypic features of international clone VI (IC-6) ST944^{Oxf}/78^{Pas} *Acinetobacter baumannii* genomes.

Strain	Isolation year	Isolation data (ward/hospital/country)	Isolate source	MLST (Oxford/Pasteur)	Antimicrobial resistance genes/mutations
AB4332[a]	16 Oct. 2016	TRM/HGR/Brazil	Tracheal secretion	ST944/78	*bla*$_{OXA-90}$, *bla*$_{OXA-72}$, IS*15DIV*–*bla*$_{CTX-M-115}$–IS*15DIV*, IS*Aba1*–*bla*$_{ADC-152}$, *aadA5*, *aac(3″)-IIa*, *aph(3′)-Ia*, *armA*, *aadB*, *msrE*, *bla*$_{TEM-1}$, *qacEΔ1*, *sul1*, GyrA (Ser83Leu), ParC (Ser80Leu)
AB5375[a]	29 Dec. 2016	ICU/HGR/Brazil	Tracheal secretion	ST944/78	*bla*$_{OXA-90}$, *bla*$_{OXA-72}$, IS*15DIV*–*bla*$_{CTX-M-115}$–IS*15DIV*, IS*Aba1*–*bla*$_{ADC-152}$, *aadA5*, *aac(3″)-IIa*, *aph(3′)-Ia*, *armA*, *aadB*, *msrE*, *bla*$_{TEM-1}$, *qacEΔ1*, *sul1*, GyrA (Ser83Leu), ParC (Ser80Leu)
AB3909	11 May 2007	Italy	Bronchial aspirate	ST944/78	*aadB*, *sul1*, *aph(3′)-Ia*, *bla*$_{OXA-90}$, IS*Aba1*-*bla*$_{ADC-152}$, *floR*, *bla*$_{OXA-58}$, *ant(3″)-II*, GyrA (Ser83Leu), ParC (Ser80Leu)

MLST, multilocus sequence typing; TRM, traumatology unit; HGR, General Hospital of Roraima; ICU, intensive care unit.
[a] Isolates from the current study.

embedded in the chromosome, also harboured phage-related and adhesion/biofilm formation genes (*big-1* and *bap*) from the intimin/invasin protein family, which are involved in host cell attachment/invasion. Genes related to adaptive traits (*mutT*), DNA repair (*umuD*), stress tolerance (*nirD*) and pathogenicity (*mgtC*, *pilB* and peptidase-coding genes) were also present.

The genomic features of the unique Italian IC-6 genome (strain 3909 recovered in 2007) available in GenBank (accession no. **GCA_000189695.2**) [1] was also assessed in order to compare it with the Brazilian IC-6 genomes (recovered in 2016). They shared the ARGs *aadB*, *sul1*, *aph(3′)-Ia*, *bla*$_{ADC-152}$ and *bla*$_{OXA-90}$ as well as the adaptation-related *mutT*, *nirD*, *mgtC*, *pilB* and peptidase-coding genes. Exceptions were *umuD*, *bla*$_{OXA-72}$ and *bla*$_{CTX-M-115}$, which were exclusively found in IC-6 Brazilian genomes. In this case, *bla*$_{CTX-M-115}$ and *bla*$_{OXA-72}$ could have been acquired by the Brazilian IC-6 strains by mobilisation events mediated by IS*15DIV* and XerC/D, respectively. Fluoroquinolone resistance-associated mutations were also observed in GyrA (Ser83Leu) and ParC (Ser80Leu) deduced proteins of Brazilian and Italian genomes (Table 2).

Spatiotemporal heterogeneity of the OXA-type genes in IC-6 strains identified in several countries since 2006 has been observed. The *bla*$_{OXA-58}$ gene characterised the ST944^{Oxf}/78^{Pas} strains recovered between 2006–2010 in Italy [1], being posteriorly replaced by *bla*$_{OXA-23}$ in that same country. This allele was the same as identified among the IC-6 strains circulating in French Guiana between 2008–2014 [9]. However, different from the IC-6 strains occurring in the aforementioned countries that harboured *bla*$_{OXA-58}$ or *bla*$_{OXA-23}$, the OXA carbapenemase gene identified in IC-6 from the USA (2009), Kuwait (2011–2012), Russia (2012–2015), Germany (2013) and Greece (2015) [4–8] was *bla*$_{OXA-72}$ from the *bla*$_{OXA-40-like/OXA-24-like}$ family, as found in the Brazilian strains described in the current study. The Brazilian strains also shared *bla*$_{CTX-M-115}$ with IC-6/ST944^{Oxf}/78^{Pas} from Russia/Germany and the USA [4,6,8]. However, the *bla*$_{CTX-M-115}$ found in Brazil was associated with IS*15DIV*, whilst in strains from Russia/Germany it was associated with IS*Ecp1* [6]. Therefore, these results altogether indicated that the Brazilian IC-6/ST944^{Oxf}/78^{Pas} strains would have arisen due to gain/loss of mobile genetic elements harbouring such ARGs.

This study revealed the emergence of the IC-6/ST944^{Oxf}/78^{Pas} in a clinical setting of Boa Vista (Roraima, Brazil), which has been persisting for ≥1 year. Interestingly, these Brazilian strains shared the *bla*$_{OXA-72}$ and *bla*$_{CTX-M-115}$ genes with IC-6/ST944^{Oxf}/78^{Pas} strains recovered in a distinct spatiotemporal context (USA, 2009; Russia, 2016) [4,6,8], which points to a direct epidemiological link among them. Moreover, besides the XDR phenotype, the Brazilian IC-6 strains presented genetic determinants related to adaptive traits, adhesion and biofilm formation, which corroborates the high biofilm growth capability that characterises this lineage and contributes to its spread and persistence globally [3].

Therefore, this study highlights the importance of epidemiological surveillance of XDR *A. baumannii* strains even outside of densely populated cosmopolitan regions in order to reveal the dispersion/occurrence of such high-risk pandemic lineages owing to their exceptional versatility and threat to public health.

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Competing interests

None declared.

Ethical approval

Not required.

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