PLOS ONE

RESEARCH ARTICLE

Molecular characterization and clonal dynamics of nosocomial *bla*$_{OXA-23}$ producing XDR *Acinetobacter baumannii*

Sabrina Royer[1]*, Paola Amaral de Campos[1], Bruna Fuga Araújo[1], Melina Lorraine Ferreira[1], Iara Rossi Gonçalves[1], Deivid William da Fonseca Batistão[2], Rebecca Tavares e Silva Brígido[3], Louise Teixeira Cerdeira[4], Luiz Gustavo Machado[1], Cristiane Silveira de Brito[1], Paulo Pinto Gontijo-Filho[1], Rosineide Marques Ribas[1]

1 Institute of Biomedical Sciences (ICBIM), Laboratory of Molecular Microbiology, Federal University of Uberlandia, Uberlandia, Minas Gerais, Brazil, **2** School of Medicine (FAMED), Federal University of Uberlandia, Uberlandia, Minas Gerais, Brazil, **3** National Reference Center for Sanitary Dermatology and Leprosy (CREDESH) Clinical Hospital Federal University of Uberlândia, Uberlândia, Minas Gerais, Brazil, **4** Department of Clinical Analysis, School of Pharmacy, University of São Paulo, São Paulo, Brazil

* sabriroyer@yahoo.com.br

Check for updates

OPEN ACCESS

Citation: Royer S, de Campos PA, Araújo BF, Ferreira ML, Gonçalves IR, Batistão DWdF, et al. (2018) Molecular characterization and clonal dynamics of nosocomial *bla*$_{OXA-23}$ producing XDR *Acinetobacter baumannii*. PLoS ONE 13(6): e0198643. https://doi.org/10.1371/journal.pone.0198643

Editor: Feng Gao, Tianjin University, CHINA

Received: March 13, 2018

Accepted: May 22, 2018

Published: June 11, 2018

Copyright: © 2018 Royer et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The nucleotide sequence data underlying this study have been uploaded to GenBank under the accession numbers NKKO00000000 (Ab13) and NKKP00000000 (Ab15).

Funding: We thank FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support and scholarships. The funders

Abstract

The emergence of infections associated to new antimicrobial resistance in *Acinetobacter baumannii* (Ab) genotypes represents a major challenge. In this context, this study aimed to determine the diversity of resistance mechanisms and investigate clonal dissemination and predominant sequence types (STs) in multidrug-resistant Ab strains of clinical (tracheal aspirate, n = 17) and environmental (surface, n = 6) origins. Additionally, the major clones found in clinical (A) and environmental (H) strains had their complete genomes sequenced. All strains were submitted to polymerase chain reactions (PCR) for the detection of the IS*Aba1*/*bla*$_{OXA-51-like}$ and IS*Aba1*/*bla*$_{OXA-23-like}$ genes, while the expression of genes encoding the *carO* porin, AdeABC (*adeB*), AdeFGH (*adeG*), and AdeIJK (*adeJ*) efflux pumps was determined by real time PCR (qPCR). Most of the strains were characterized as extensively drug-resistant (XDR) with high minimal inhibitory concentrations (MICs) detected for tigecycline and carbapenems. Associations between IS*Aba1*/OXA-51 and IS*Aba1*/OXA-23 were observed in 91.3% and 52.2% of the strains, respectively. Only the *adeB* gene was considered hyper-expressed. Furthermore, most of the strains analyzed by the MuLtilocus Sequence-Typing (MLST) were found to belong to the clonal complex 113 (CC113). In addition, a new ST, ST1399, belonging to CC229, was also discovered herein. Strains analyzed by whole genome sequencing presented resistance genes linked to multidrug-resistance phenotypes and confirmed the presence of Tn*2008*, which provides high levels carbapenem-resistance.

had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Acinetobacter baumannii (Ab) is one of the most successful pathogens responsible for hospital-acquired infections worldwide, mainly in immunocompromised patients and those admitted to intensive care units (ICUs) [1,2].

Carbapenem resistance in Ab is most frequently mediated by intrinsic (OXA-51-like) or acquired (mainly OXA-23-like) oxacillinases [3]. The hyper-expression of oxacillinases is often associated to insertion sequences (ISs), such as the IS*Aba1*sequence, located upstream of the OXA genes [4–6]. In addition, the increased expression of chromosomal genes of resistance-nodulation-cell division (RND)-type efflux systems [2,7] and the decreased expression of certain outer membrane channel-forming proteins [8,9] play an important role in Ab multidrug resistance. The relative contribution of these resistance mechanisms remains poorly assessed in clinical contexts [8].

Regarding the clonal nature and the spread of different sequence types (STs), both Brazilian and Latin American studies conducted between 2011 and 2016 demonstrated that the most widespread Ab strains belong to CC113 and CC109 [10–16], characterized by MuLtilocus Sequence-Typing—University of Oxford (MLST-UO). Other studies worldwide indicate that multidrug-resistant Ab is related to CC92 [17,12,18–19]. Tracking the evolution and clonal dissemination of carbapenem resistance in Ab isolates is important to support the implementation of control strategies, which is only possible through a comprehensive understanding of the complete genome of these strains.

In this context, the aim of the present study was to investigate the clonal dissemination and genetic basis of resistance among multidrug-resistant Ab strains recovered from an adult ICU in Brazil. Additionally, one clinical and one environmental strain belonging to the prevalent clones had their complete genomes sequenced.

Material and methods

Bacterial strains and setting

The origin and epidemiological characteristics of the strains used herein are described in Table 1. Clinical and environmental *A*. *baumannii* isolates were obtained from patients with ventilator-associated pneumonia (VAP) and the environments around their beds (bedside table, bed rail and door knob), respectively. The strains were obtained from April 2011 to June 2012, in a 30-bed clinical-surgical intensive care unit (ICU) at the Clinical Hospital belonging to the Federal University of Uberlandia, Minas Gerais, Brazil. The 23 isolates investigated herein were selected according to their resistance profile and pulsotype, obtained through Pulsed Field Gel Electrophoresis (PFGE), as published previously [20].

Clinical microbiology

Resistance to tigecycline, imipenem and meropenem was determined by the Etest® method according to the manufacturer's guidelines (AB Biodisk, Solna, Sweden). To confirm the results of Etest® for tigecycline, the broth microdilution technique was performed. All the resistance tests and the quality control protocols were done in accordance with the Clinical and Laboratory Standards Institute recommended practices [21]. Since there were no breakpoints available for tigecycline for *Acinetobacter* spp., US Food and Drug Administration (FDA) tigecycline breakpoints listed for Enterobacteriaceae ($\leq$ 2, 4 and $\geq$ 8 μg/ml for susceptible, intermediate and resistant strains, respectively) were applied. Multidrug-

Table 1. Molecular characterization by polymerase chain reaction (PCR) of resistance determinants and distribution of MICs to carbapenems and tigecycline in 23 strains of *A. baumannii* recovered from endotracheal aspirate and environment in an adult intensive care unit.

Strains[1]/ Source	Resistance genes	Porines genes	Efflux pumps genes	MDR[2]/ XDR[3]	MIC[4] (μg/m) IPM[5]/ MEM[6]	MIC (μg/mL) TGC[7]	MIC[8] (μg/mL) TGC	PFGE[9] profile
13/EA[10]	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	>256	64	A
15/EN[11]	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	96	64	H
17/EN	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	48	64	H
20/EN	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	MDR	>32/>32	24	64	E
2/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	24/>32	>256	64	A
16/EN	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	24/>32	96	64	H
18/EN	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	16/>32	64	64	G
19/EN	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	12/32	48	64	H
1/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	12/16	>256	64	A
9/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	MDR	4/3	8	64	C
5/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1/OXA-23; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	8/12	48	64	D
3/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	>256	64	A
12/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-51*	*omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	24	64	C
8/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	24/32	64	64	A
6/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	16/24	256	64	A
11/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	8/6	>256	64	A
14/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1/OXA-23*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	256	64	G
4/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	>256	64	A
10/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1/OXA-51*	*omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	>32/>32	32	32	B
7/EA	*bla*$_{OXA-51}$; *bla*$_{OXA-23}$; *ISAba1/OXA-51*	*omp33-36*	*adeB*; *adeG*; *adeJ*	XDR	16/>32	24	32	A
23/EA	*bla*$_{OXA-51}$; *ISAba1; ISAba1/OXA-51*	*omp33-36*	*adeB*; *adeG*; *adeJ*	MDR	1/3	128	64	G
21/EA	*bla*$_{OXA-51}$; *ISAba1; ISAba1/OXA-51*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	No MDR[12]	0,75/3	6	64	G
22/EA	*bla*$_{OXA-51}$; *ISAba1*	*carO*; *omp33-36*	*adeB*; *adeG*; *adeJ*	No MDR	3/6	8	64	F

[1]All negative strains for *bla*$_{OXA-24}$, *bla*$_{OXA-58}$ and *bla*$_{OXA-143}$ genes;

[2]MDR, Multidrug-resistant;

[3]XDR, Extensively drug-resistant;

[4]MIC, Minimum Inhibitory Concentration—Etest®;

[5]IPM, Imipenem (0,002–32μg/mL);

[6]MEM, Meropenem (0,002–32μg/mL);

[7]TGC, Tigecycline (0,016–256μg/mL);

[8]MIC, Minimum Inhibitory Concentration—Broth microdilution (0,125–256μg/mL);

[9]PFGE, Pulsed Field Gel Electrophoresis;

[10]EA, Endotracheal aspirate;

[11]EN, Environment.

[12]No MDR, strains do not present resistance to three or more antimicrobial categories.

https://doi.org/10.1371/journal.pone.0198643.t001

resistant (MDR) was defined as resistance to three or more categories of antibiotics while extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, according to Magiorakos and collaborators [22].

Polymerase chain reaction

The DNA extraction was performed by thermal lysis and a conventional PCR assay was performed for 23 isolates to detect the following genes: IS*Aba1*/$bla_{\text{OXA-51-like}}$, IS*Aba1*/$bla_{\text{OXA-23-like}}$, *omp33-36*, *carO* (29 kDa), *adeB*, *adeG* and *adeJ*, using previous published primers (S1 Table) [23,4,24,25,26,2]. The amplified PCR products were visualized by electrophoresis on 1.5% agarose gels using the photo documentation System L-Pix EX (Loccus Biotechnology, Brazil).

Relative quantification of CarO porin and *adeB*, *adeG* and *adeJ* efflux system genes by real time PCR (qPCR)

Out of a total of 23 strains with their clonals profiles previously evaluated [20] 10 were selected for analysis by qPCR. The *carO*, *adeB*, *adeG* and *adeJ* transcription was evaluated by qPCR using Power-SYBR Green PCR Master Mix (Applied Biosystems). The single-copy housekeeping *rpoB* gene from Ab was used as endogenous gene for normalization (S1 Table) [2,27]. The relative quantification (RQ) results were presented as ratios of normalized target gene transcription between the Ab isolates and the Type Strain ATCC 19606 (calibrator), which were obtained according to the following equation: RQ = 2-ΔΔCT, where CT is the value corresponding to the crossing point of the amplification curve with the threshold; ΔCT = target CT or calibrator CT–endogenous CT; and ΔΔCT = target ΔCT—calibrator ΔCT. Reduced *carO* differential transcription of strains relative to that of ATCC 19606 was considered significant when the ratios obtained between RQ values (RQ value of calibrator/RQ value of strains) were $\geq$2.0 [28], and the overexpression of *adeB*, *adeG* and *adeJ* was considered significant when the ratios obtained between RQ values were $\geq$4.0 [2]. Each experiment was performed in triplicate in two independent assays.

Multilocus Sequence Typing (MLST)

The same 10 strains selected for the qPCR reaction were selected for genotyping by MultiLocus Sequence Typing (MLST) as described [29]. The methodology was carried out following the guidelines of the website <https://pubmlst.org/abaumannii/info/primers_Oxford.shtml>. The sequence of amplified internal fragments of housekeeping genes *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD* was determined and compared with those in the Ab MLST database of Oxford scheme [29]. Clonal complexes (CCs) were formed by Sequence Types (STs) with five or more identical alleles by goeBURST (goeburst.phyloviz.net).

Whole-genome sequencing

Two strains, one clinical and other environmental, representing prevalent clones previously evaluated by PFGE, were selected for whole-genome sequencing. The total genomic DNA of selected isolates was sequenced, using Illumina NexSeq 500 sequencer (Illumina, San Diego, CA), and the sequence reads were de novo assembled using Velvet pipeline. The pairwise alignment was performed by BLASTn homology searches (https://blast.ncbi.nlm.nih.gov), and in silico comparative analysis using the Center for Genomic Epidemiology (CGE) pipelines. The amino acid sequences of the CarO were aligned using ClustalW with sequences available from GenBank.

Statistical analysis

Statistical analyses were performed using GraphPad Prism v.5 (GraphPad Software, San Diego, CA). Quantitative assays were compared using one-way analysis of variance (ANOVA)

and Bonferroni multiple comparison test. All tests were performed with a confidence level of 95% and statistical significance was defined as $P \leq 0.05$.

Ethical considerations

The data and the samples analyzed in the present study were obtained in accordance with the norms and approved by the Federal University of Uberlandia Ethics Committee (UFU), through license number 228/11.

Results

The majority (18/23; 78.3%) of strains included in this study were XDR. The presence of associations between IS*Aba1*/OXA-51 and IS*Aba1*/OXA-23 was observed in 21 (91.3%) and 12 (52.2%) strains, respectively. All analyzed environmental strains displayed the IS*Aba1* insertion element linked to both the $bla_{\text{OXA-51}}$ and the $bla_{\text{OXA-23}}$ genes (Table 1).

The presence of outer membrane proteins (OMP) genes *carO* and *omp33-36*, were detected at 82.6% (19/23) and 100%, frequencies, respectively. The presence of genes encoding the AdeABC (*adeB*), AdeFGH (*adeG*), and AdeIJK (*adeJ*) efflux pumps was evidenced in all analyzed strains. Most exhibited very high MICs for both carbapenems and tigecycline when evaluated by the Etest® method. For the latter antimicrobial drug, the results were confirmed by broth microdilution, and all strains were classified as resistant (Table 1).

The expression of the *adeB*, *adeG*, and *adeJ* genes, which encode RND type efflux systems components, and the gene encoding the OMP of 29 kDa (*carO*), were measured by qPCR. The relative gene expression of the tested strains compared to the reference is displayed Fig 1. Based on the transcription levels determined as cut-offs for the hyperexpression of the efflux systems and decreased OMP expression, only the *adeB* gene, which codifies the production of the AdeABC efflux pump, was considered as hyper-expressed. Significant differences in the expression levels of most of the strains in relation to the *adeB* gene were observed (Fig 1B), while the opposite was detected when analyzing the *carO*, *adeG*, and *adeJ* genes (Fig 1A, 1C and 1D).

MLST revealed eight STs and five CCs (Fig 2), distributed as follows: 1) CC113, ST227 (n = 2), ST233 (n = 1), and ST258 (n = 1); 2) CC229, ST1399, and ST1489; 3) CC109 and ST405 (n = 2); 4) ST/CC235; and 5) ST/CC231. ST1399 is described for the first time herein and was deposited in the MLST database (https://pubmlst.org/bigsdb?db=pubmlst_abaumannii_oxford_seqdef). The three environmental strains included in this analysis belonged to distinct CCs: CC229, CC113, and CC109. CC109 and CC113 were the only CCs presenting strains with the same ST in both environmental and clinical specimens.

The total genome belonging to the Ab13 and Ab15 strains exhibited sizes of 3.76 Mb and 3.69 Mb, respectively, and generated a total of 7,694,708 and 9,944,440 reads, respectively, yielding approximately 290X and 370X sequence coverages. The whole genome sequencing analysis confirmed that the Ab13 strain (tracheal aspirate, clone A) belongs to ST/CC231, while the Ab15 strain (environmental, clone H) belongs to ST405/CC109. Resistance to carbapenems was explained by the presence of the Tn*2008* (IS*Aba1*-$bla_{\text{OXA-23}}$) transposon, located in plasmids (Fig 3A). Both strains exhibited the $bla_{\text{OXA-69}}$ gene, a variant of the intrinsically encoded $bla_{\text{OXA-51-like}}$ gene, while third-generation cephalosporin resistance occurs by increased transcription of the *ampC* gene ($bla_{\text{ADC-25}}$) when associated with IS*Aba1* (Fig 3B).

In the Ab13 strain, resistance to aminoglycosides is justified by the presence of an enzyme that modifies this drug (*aacA4*). In the Ab15 strain, the genes associated to aminoglycoside resistance are *aacA4*, *strA*, and *strB*, and *floR* and *sul2* for chloramphenicol and sulfonamides,

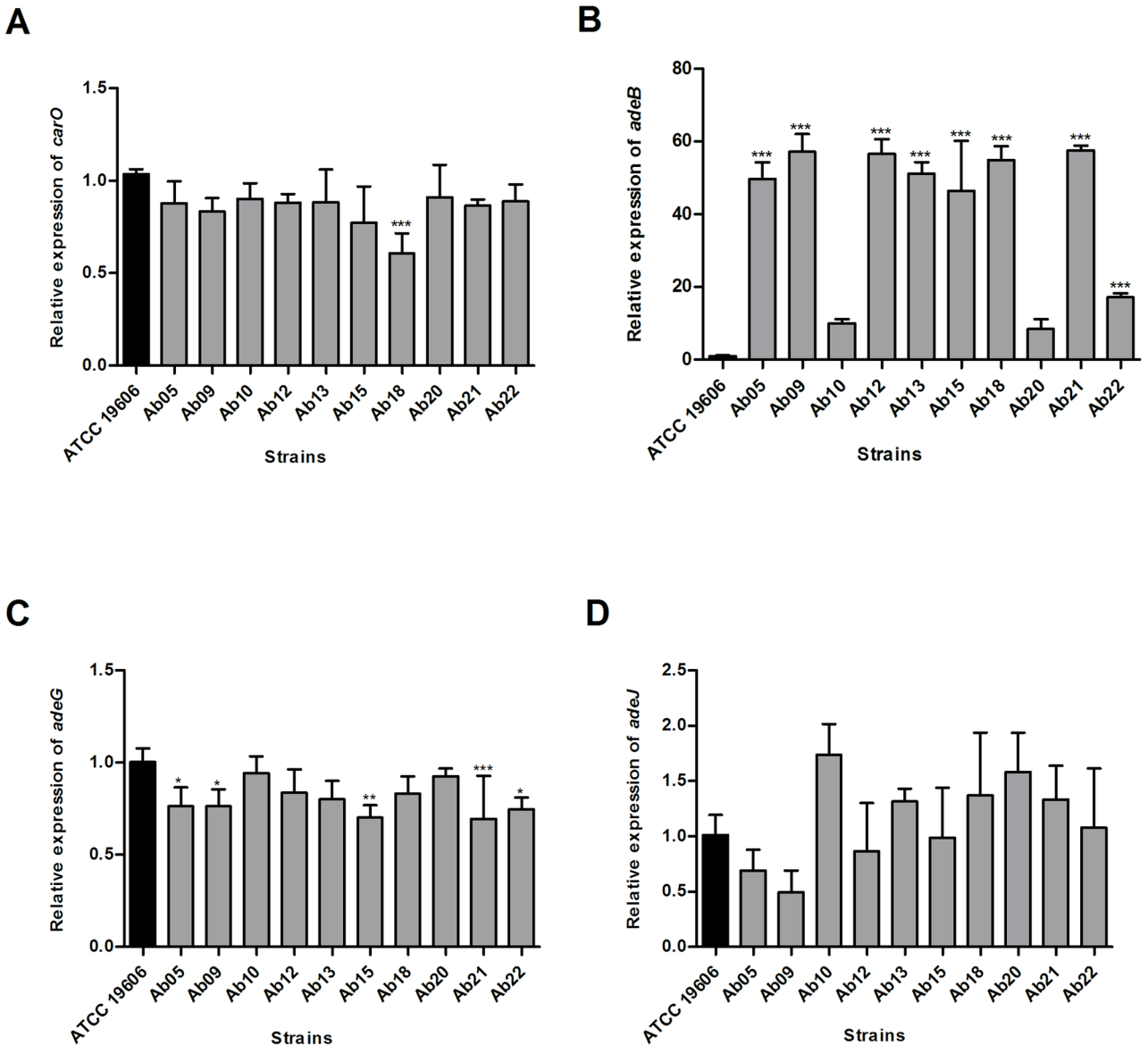

Fig 1. Relative gene expression of the *carO* gene and the genes related to the three efflux pumps (AdeABC, AdeFGH and AdeIJK), determined by qPCR. The results are shown in relation to strain ATCC 19606 used as reference. Each sample was tested in triplicate in two independent assays. Results represent means plus standard deviation (error bars). $^{*}P<0.01$; $^{**}P<0.001$; $^{***}P<0.0001$, using one-way ANOVA and Bonferroni multiple comparison test.

https://doi.org/10.1371/journal.pone.0198643.g001

respectively. The plasmid-mediated quinolone resistance gene (PMQR) *aac(6')Ib-cr*, was identified in both strains.

Sequencing of the *carO* gene revealed that both analyzed strains exhibit an isoform protein different from those already registered in the NCBI database (CarOa and CarOb) but with a similarity of 99% to the SJ22 strain registered by Sen and Joshi [30] (Accession number in GenBank KP658474.1), differing only in a change at position 218 (substitution of glutamine for lysine, Q218K) (Fig 4). The strains displayed 93% identity when compared to the reference ATCC 19606 strain (CarOa; accession number in GenBank KP658473), which is susceptible to

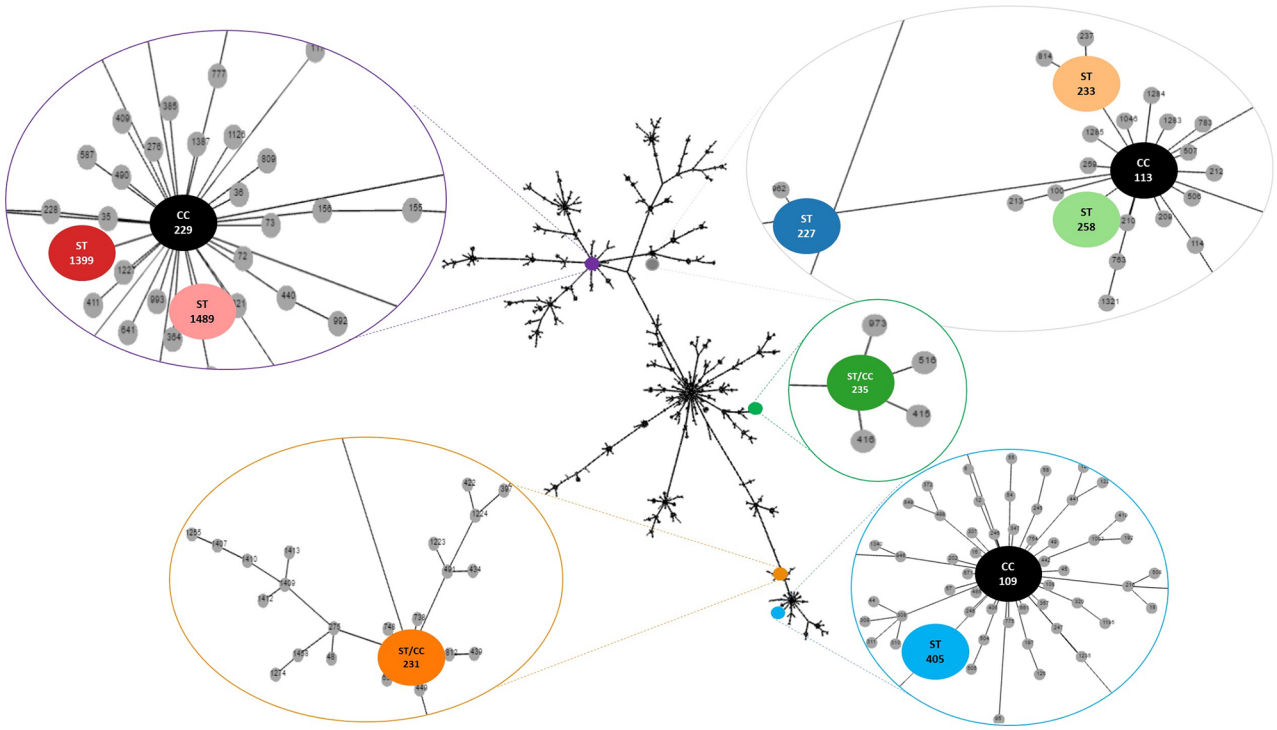

Fig 2. Diagram constructed using the goeBURST algorithm and displayed on phyloviz software (PHYLOVIZ Online) indicating the similarity among sequence types (STs). The Clonal Complexes (CCs) and STs observed in the present study are enlarged and highlighted by color.

https://doi.org/10.1371/journal.pone.0198643.g002

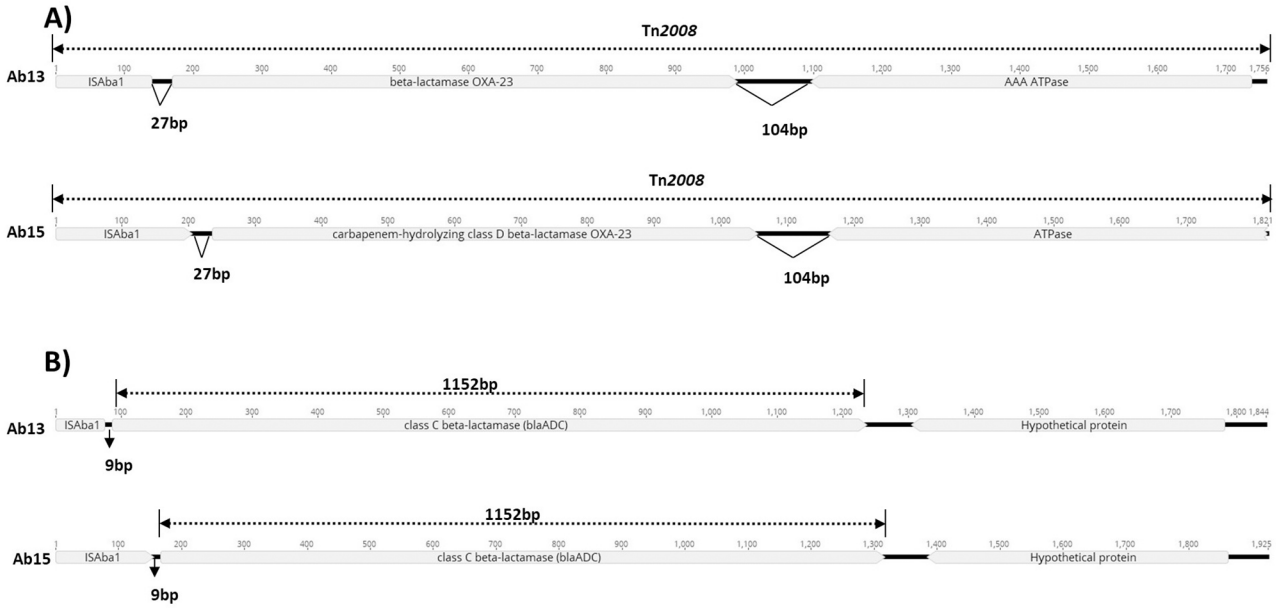

Fig 3. (A) Schematic representation of Tn*2008* with IS*Aba1* located upstream of the $bla_{\text{OXA-23}}$ gene in the analyzed strains. (B) Schematic representation of the IS*Aba1* localization upstream to the $bla_{\text{ADC-25}}$ gene.

https://doi.org/10.1371/journal.pone.0198643.g003

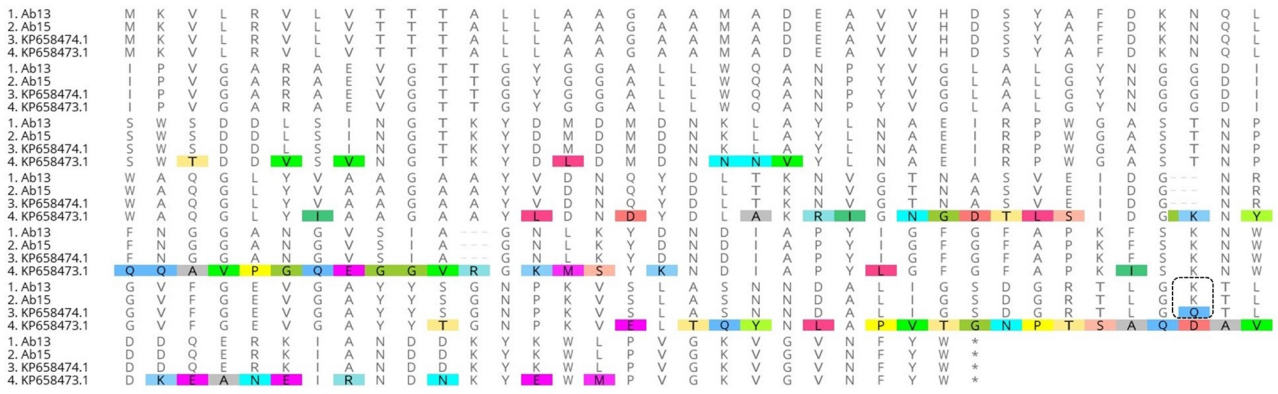

Fig 4. Sequence alignment of *carO* gene for two representative *Acinetobacter baumannii* strains from this study (Ab13 and Ab15) with SJ22 (GenBank: KP658474.1) and ATCC 19606 (GenBank: KP658473.1) strains, indicating homology and difference in amino acids. The alignment was performed using ClustalW. The dotted line indicates the point mutation region (Q218K).

https://doi.org/10.1371/journal.pone.0198643.g004

carbapenems due to the presence of point mutations. In addition, no genetic disruption by IS*Aba1* insertion was observed; therefore, no protein inactivation occurred.

Discussion

Resistance to antibiotics in Ab has reached alarming levels worldwide, particularly for carbapenems, and strains have been shown to be susceptible only to polymyxins [31–35]. In Brazil, unfortunately, resistance rates to carbapenems are very high (80.7%) [36] and, according to a study carried out by Rossi et al. (2017)[37], a variation of 30 to 70% in resistance to carbapenems was detected in *Acinetobacter* species between 2010 and 2014.

The increase in resistance to carbapenems in clinical Ab strains is mainly associated to the dissemination of OXA-23-producing strains [38–43,26,44], which can be explained by the fact that this gene can also be allocated in plasmids, partly justifying its global reach [45–48]. Allied to this, the presence of specific ISs, such as IS*Aba1*, located adjacent to the bla_{OXA} genes, leads to an increase of their expression, resulting in a further decrease in susceptibility to carbapenems [49,4,50–52].

In the present study, approximately half of the analyzed strains were associated to IS*Aba1*/OXA-23, related to high MICs for carbapenems. Viana et al. (2016) [53] observed similar data (i.e., an increase in this association from 22 to 73% between 2009 and 2013, also correlated to elevated MICs for carbapenems. In some of the evaluated strains, no association with either IS*Aba1*/OXA-51 or IS*Aba1*/OXA-23 was observed, despite high MICs, which can be justified by the coexistence of other resistance mechanisms in these strains. These results suggest that the IS*Aba1*/OXA-23 combination may be one of the most significant resistance mechanisms in Ab associated to high XDR phenotypes frequencies and high MICs for carbapenems.

Carbapenem resistance in Ab [54–55] may also result from modifications in the primary structure or loss of outer membrane proteins (OMPs) (porins), such as the 33–36 kDa and the 29 kDa protein named CarO [9]. In the case of OMP CarO, modifications are mostly a result from the rupture of the gene by several insertion elements [56]. From these results, we can infer that the CarO protein is not responsible for carbapenem resistance in the evaluated strains, since no reduced expression levels of this protein were observed. However, the sequence analysis of the *carO* gene revealed a protein isoform different from those already registered in the NCBI database (CarOa and CarOb). A point mutation in the protein (Q218K)

has not been reported so far, so it was not possible to determine its exact participation in imipenem resistance.

Another antimicrobial resistance mechanism present in this microorganism is the hyperexpression of efflux pumps, such as those belonging to the RND family (AdeABC, AdeFGH, and AdeIJK) [8]. In addition, AdeABC and AdeFGH play an important role in acquired resistance [57,2], while AdeIJK contributes to intrinsic resistance [58]. Only the *adeB* gene was hyper-expressed based on the qPCR results. According to the literature [7], strains containing the AdeABC pump confer resistance to various antibiotics, including most β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, tigecycline, macrolides, lincosamides, and chloramphenicol. Thus, alongside the presence of IS*Aba1*/OXA-23, this pump is another important mechanism present in the strains analyzed herein.

Tigecycline is an interesting therapeutic option to treat infection by carbapenem-resistant Gram-negative bacteria [59]. However, the isolates evaluated herein demonstrated resistance to this antimicrobial by the Etest® and microdilution methods. A confirmatory evaluation of MICs $\geq$ 2 μg/ml determined by Etest® is mandatory, as these MICs values could be up to four-fold higher than those obtained by the microdilution method [60,61,62]. The results reported herein corroborate these findings and confirm tigecycline resistance of the strains investigated in this study.

In addition to the genes responsible for carbapenem resistance and high MIC values for tigecycline, most of the strains analyzed by MLST belonged to CC113, confirming its dissemination in Brazil and Latin America [12,63,13–14,64,15–16]. Additionally, a new ST, ST1399, belonging to CC229, is reported. In Brazil, deserves attention another CC identified in carbapenem-resistant Ab strains, CC109, corresponding to the international clone 1 [11,12,64].

Whole genome sequencing of the Ab13 and Ab15 strains demonstrated that carbapenem-resistance is mainly due to the presence of Tn*2008*, which is easily propagated among strains and includes different clones (as evidenced herein) disseminated worldwide [65,66]. In addition, the intrinsic resistance to third generation cephalosporins is due to the increased transcription of the $bla_{\text{ADC-25}}$ gene, due to the presence of IS*Aba1*, adjacent to the gene, acting as a strong promoter, as described in the literature [67–68,5]. Another detected mechanism was the presence of genes encoding aminoglycoside-modifying enzymes, such as *aacA4* [68,5]. In addition, the PMQR gene *aac(6')Ib-cr* was also identified. This is relevant data, since this gene is well described in Enterobactericeae family members and in *Pseudomonas aeruginosa* [69–71], but has seldom been studied in Ab [72].

Conclusions

Although a new ST was detected herein in Ab, the present study also observed a wide variety of CCs related to XDR strains carrying the $bla_{\text{OXA-23}}$ gene, frequently associated with IS*Aba1* (Tn*2008*) as the main carbapenem-resistance mechanism, in addition to the hyperexpression of the AdeABC efflux pump. Understanding resistance mechanisms and the pathogenic potential of this microorganism, both from environmental and clinical origins, aids in explaining its persistence in the hospital environment and can provide tools to improve the treatment of serious infections, as well as increase control and prevention regarding these infections.

Nucleotide sequence accession numbers

The nucleotide sequence data underlying this study have been uploaded to GenBank under the accession numbers NKKO00000000 (Ab13) and NKKP00000000 (Ab15).

Supporting information

S1 Table. Primer sequences of genes confirmed with PCR and qPCR.
(DOCX)

Acknowledgments

The authors wish to thank members of Laboratory of Bacterial Resistance and Therapeutic Alternatives, Department of Microbiology—ICBII / USP, especially PhD Nilton Licopan for assisting in the analysis of sequencing. We are grateful to PhD Ana Paula Guedes Frazzon (Federal University of Rio Grande do Sul) who kindly provided the control strain *A. baumannii* ATCC 19606. We thank FAPEMIG (Fundação de Amparo à Pesquisa de Minas Gerais), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support and scholarships.

Author Contributions

Conceptualization: Sabrina Royer, Rosineide Marques Ribas.

Formal analysis: Sabrina Royer.

Investigation: Sabrina Royer.

Methodology: Sabrina Royer, Paola Amaral de Campos, Bruna Fuga Araújo, Melina Lorraine Ferreira, Iara Rossi Gonçalves, Rebecca Tavares e Silva Brígido, Louise Teixeira Cerdeira, Luiz Gustavo Machado, Cristiane Silveira de Brito.

Supervision: Paulo Pinto Gontijo-Filho, Rosineide Marques Ribas.

Visualization: Paola Amaral de Campos, Deivid William da Fonseca Batistão.

Writing – original draft: Sabrina Royer, Paola Amaral de Campos, Deivid William da Fonseca Batistão, Paulo Pinto Gontijo-Filho, Rosineide Marques Ribas.

Writing – review & editing: Sabrina Royer, Paulo Pinto Gontijo-Filho, Rosineide Marques Ribas.

References

1. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of *Acinetobacter baumannii*: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol. 2017: 13:7–55.
2. Yoon EJ, Courvalin P, Grillot-Courvalin C. RND-type efflux pumps in multidrug-resistant clinical isolates of *Acinetobacter baumannii*: major role for AdeABC overexpression and AdeRS mutations. Antimicrob Agents Chemother. 2013: 57:2989–2995. https://doi.org/10.1128/AAC.02556-12 PMID: 23587960
3. Evans BA, Amyes SG. OXA β-lactamases. Clin Microbiol Rev. 2014: 27:241–263. https://doi.org/10.1128/CMR.00117-13 PMID: 24696435
4. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of IS Aba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett. 2006: 258:72–7. https://doi.org/10.1111/j.1574-6968.2006.00195.x PMID: 16630258
5. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev. 2008: 21:538–82. https://doi.org/10.1128/CMR.00058-07 PMID: 18625687
6. Pagano M, Martins AF, Machado ABMP, Barin J, Barth AL. Carbapenem-susceptible *Acinetobacter baumannii* carrying the ISAba1 upstream blaOXA-51-like gene in Porto Alegre, southern Brazil. Epidemiol Infect. 2013: 141:330–3. https://doi.org/10.1017/S095026881200074X PMID: 22717017

7. Yoon EJ, Chabane YN, Goussard S, Snesrud E, Courvalin P, Dé E, et al. Contribution of resistance-nodulation-cell division efflux systems to antibiotic resistance and biofilm formation in *Acinetobacter baumannii*. MBio. 2015: 6:e00309–15. https://doi.org/10.1128/mBio.00309-15 PMID: 25805730
8. Vila J, Martí S, Sánchez-Céspedes J. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J Antimicrob Chemother. 2007: 59:1210–5. https://doi.org/10.1093/jac/dkl509 PMID: 17324960
9. Novovic K, Mihajlovic S, Vasiljevic Z, Filipic B, Begovic J, Jovcic B. Carbapenem-resistant *Acinetobacter baumannii* from Serbia: Revision of CarO classification. PloS One. 2015: 10:e0122793. https://doi.org/10.1371/journal.pone.0122793 PMID: 25822626
10. Grosso F, Carvalho KR, Quinteira S, Ramos A, Carvalho-Assef APDA, Asensi M D, et al. OXA-23-producing *Acinetobacter baumannii*: a new hotspot of diversity in Rio de Janeiro? J Antimicrob Chemother. 2011: 66:62–5. https://doi.org/10.1093/jac/dkq406 PMID: 21051372
11. Martins N, Martins IS, Freitas WV, Matos JA, Magalhães ACG, Girão VBC, et al. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant *Acinetobacter baumannii*. Transplant Infectious Disease, 2012: 14:316–320. https://doi.org/10.1111/j.1399-3062.2011.00701.x PMID: 22168176
12. Clímaco EC, de Oliveira ML, Pitondo-Silva A, Oliveira MG, Medeiros M, Lincopan N, et al. Clonal complexes 104, 109 and 113 playing a major role in the dissemination of OXA-carbapenemase-producing *Acinetobacter baumannii* in Southeast Brazil. Infect Genet Evol. 2013: 19:127–33. https://doi.org/10.1016/j.meegid.2013.06.024 PMID: 23838284
13. Martins N, Martins IS, de Freitas WV, de Matos JA, Girão VBDC, Coelho-Souza T, et al. Imported and intensive care unit-born *Acinetobacter baumannii* clonal complexes: one-year prospective cohort study in intensive care patients. Microb Drug Resist. 2013: 19:216–23. https://doi.org/10.1089/mdr.2012.0174 PMID: 23336529
14. Stietz MS, Ramírez MS, Vilacoba E, Merkier AK, Limansky AS, Centrón D, Catalano M. *Acinetobacter baumannii* extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III. Infect Genet Evol. 2013: 14:294–301. https://doi.org/10.1016/j.meegid.2012.12.020 PMID: 23313831
15. Ramirez MS, Montaña S, Cassini M, Centron D. Preferential carriage of class 2 integrons in *Acinetobacter baumannii* CC113 and novel singletons. Epidemiol Infect. 2015: 143:3118–21. https://doi.org/10.1017/S0950268815000060 PMID: 25697643
16. Leite GC, Oliveira MS, Perdigão-Neto LV, Rocha CKD, Guimarães T, Rizek C, et al. Antimicrobial combinations against pan-resistant *Acinetobacter baumannii* isolates with different resistance mechanisms. PloS One, 2016: 11: e0151270. https://doi.org/10.1371/journal.pone.0151270 PMID: 26998609
17. 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: 15:237–47. https://doi.org/10.1016/j.drup.2012.06.001 PMID: 22841809
18. Medeiros M, Lincopan N. Oxacillinase (OXA)-producing *Acinetobacter baumannii* in Brazil: clinical and environmental impact and therapeutic options. J. Bras. Patol. Med. Lab. 2013: 49:391–405.
19. Huang G, Yin S, Gong Y, Zhao X, Zou L, Jiang B, et al. Multilocus sequence typing analysis of carbapenem-resistant *Acinetobacter baumannii* in a Chinese burns institute. Front Microbiol. 2016: 7:1717. https://doi.org/10.3389/fmicb.2016.01717 PMID: 27881972
20. Royer S, Faria ALS, Seki LM, Chagas TPG, de Campos PA, Batistão DWF, et al. Spread of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clones in patients with ventilator-associated pneumonia in an adult intensive care unit at a university hospital. Braz J Infect Dis. 2015: 19:350–7. https://doi.org/10.1016/j.bjid.2015.03.009 PMID: 25997783
21. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Five Informational Supplement M100-S25. CLSI, Wayne, P.A. 2015.
22. 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: 18:268–81. https://doi.org/10.1111/j.1469-0691.2011.03570.x PMID: 21793988
23. Segal H, Garny S, Elisha BG. Is IS(ABA-1) customized for *Acinetobacter*? FEMS Microbiol Lett. 2005: 243:425–9. https://doi.org/10.1016/j.femsle.2005.01.005 PMID: 15686845
24. Mostachio AK, Levin AS, Rizek C, Rossi F, Zerbini J, Costa SF. High prevalence of OXA-143 and alteration of outer membrane proteins in carbapenem-resistant *Acinetobacter* spp. isolates in Brazil. Int J Antimicrob Agents. 2012: 39:396–401. https://doi.org/10.1016/j.ijantimicag.2012.01.021 PMID: 22455794

25. Lu PL, Doumith M, Livermore DM, Chen TP, Woodford N. Diversity of carbapenem resistance mechanisms in *Acinetobacter baumannii* from a Taiwan hospital: spread of plasmid-borne OXA-72 carbapenemase. J Antimicrob Chemother. 2009: 63:641–7. https://doi.org/10.1093/jac/dkn553 PMID: 19182237

26. Lin L, Ling BD, Li XZ. Distribution of the multidrug efflux pump genes, adeABC, adeDE and adeIJK, and class 1 integron genes in multiple-antimicrobial-resistant clinical isolates of *Acinetobacter baumannii*–Acinetobacter calcoaceticus complex. Int J Antimicrob Agents. 2009: 33:27–32. https://doi.org/10.1016/j.ijantimicag.2008.06.027 PMID: 18790612

27. 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: 85:90–7. https://doi.org/10.1016/j.diagmicrobio.2016.01.018 PMID: 26971181

28. Fonseca EL, Scheidegger E, Freitas FS, Cipriano R, Vicente ACP. Carbapenem-resistant *Acinetobacter baumannii* from Brazil: role of carO alleles expression and blaOXA-23 gene. BMC Microbiol. 2013: 13:245. https://doi.org/10.1186/1471-2180-13-245 PMID: 24195496

29. Bartual SG, Seifert H, Hippler C, Luzon MAD, Wisplinghoff H, Rodríguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol. 2005: 43:4382–90. https://doi.org/10.1128/JCM.43.9.4382-4390.2005 PMID: 16145081

30. Sen B, Joshi SG. Studies on *Acinetobacter baumannii* involving multiple mechanisms of carbapenem resistance. J Appl Microbiol. 2016: 120:619–29. https://doi.org/10.1111/jam.13037 PMID: 26709119

31. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther. 2013: 11:297–308. https://doi.org/10.1586/eri.13.12 PMID: 23458769

32. Jones RN, Flonta M, Gurler N, Cepparulo M, Mendes RE, Castanheira M. Resistance surveillance program report for selected European nations (2011). Diagn Microbiol Infect Dis. 2014: 78: 429–436. https://doi.org/10.1016/j.diagmicrobio.2013.10.008 PMID: 24440509

33. Costello SE, Gales AC, Morfin-Otero R, Jones RN, Castanheira M. Mechanisms of Resistance, Clonal Expansion, and Increasing Prevalence of *Acinetobacter baumannii* Strains Displaying Elevated Tigecycline MIC Values in Latin America. Microb Drug Resist. 2016: 22: 253–258. https://doi.org/10.1089/mdr.2015.0168 PMID: 26716768

34. Jasemi S, Douraghi M, Adibhesami H, Zeraati H, Rahbar M, Boroumand MA, et al. Trend of extensively drug-resistant *Acinetobacter baumannii* and the remaining therapeutic options: a multicenter study in Tehran, Iran over a 3-year period. Lett Appl Microbiol. 2016: 63:466–472. https://doi.org/10.1111/lam.12669 PMID: 27626896

35. Gao L, Lyu Y, Li Y. Trends in Drug Resistance of *Acinetobacter baumannii* over a 10-year Period: Nationwide Data from the China Surveillance of Antimicrobial Resistance Program. Chin Med J (Engl). 2017: 130:659–664.

36. ANVISA—National Health Surveillance Agency. Newsletter—Patient Safety and Quality of Health Services. www.anvisa.gov.br/. 2014.

37. Rossi F, Girardello R, Cury AP, Di Gioia TSR, Almeida JND Jr, Duarte AJDS. Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years. Braz J Infect Dis. 2017: 21:98–101. https://doi.org/10.1016/j.bjid.2016.09.011 PMID: 27832961

38. Dalla-Costa LM, Coelho JM, Souza HA, Castro ME, Stier CJ, Bragagnolo KL, et al. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J Clin Microbiol. 2003: 41:3403–6. https://doi.org/10.1128/JCM.41.7.3403-3406.2003 PMID: 12843104

39. Carvalho KR, Carvalho-Assef APDA, Peirano G, dos Santos LCG, Pereira MJF, Asensi MD. Dissemination of multidrug-resistant Acinetobacter baumannii genotypes carrying blaOXA-23 collected from hospitals in Rio de Janeiro, Brazil. Int J Antimicrob Agents. 2009: 34:25–8. https://doi.org/10.1016/j.ijantimicag.2008.12.009 PMID: 19216059

40. Martins AF, Kuchenbecker R, Sukiennik T, Boff R, Reiter KC, Lutz L, et al. Carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme: dissemination in Southern Brazil. Infection. 2009: 37:474–6. https://doi.org/10.1007/s15010-009-9003-9 PMID: 19768380

41. Mostachio AK, van der Heidjen I, Rossi F, Levin AS, Costa SF. Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-β-lactamases in carbapenem-resistant Acinetobacter spp. J Med Microbiol. 2009: 58:1522–4. https://doi.org/10.1099/jmm.0.011080-0 PMID: 19574410

42. Higgins PG, Lehmann M, Seifert H. Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents. 2010: 35:305.

43. Chagas TPG, Carvalho KR, de Oliveira Santos IC, Carvalho-Assef APDA, 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: 79:468–72. https://doi.org/10.1016/j.diagmicrobio.2014.03.006 PMID: 24880823

44. Vasconcelos AT, Barth AL, Zavascki AP, Gales AC, Levin AS, Lucarevschi BR, et al. The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. Braz J Infect Dis. 2015: 19:350–7.

45. Brown S, Amyes S. OXA β-lactamases in *Acinetobacter*: the story so far. J Antimicrob Chemother. 2005: 57:1–3. https://doi.org/10.1093/jac/dki425 PMID: 16332731

46. Labarca JA, Salles MJC, Seas C, Guzmán-Blanco M. Carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in the nosocomial setting in Latin America. Crit Rev Microbiol. 2016: 42:276–92. https://doi.org/10.3109/1040841X.2014.940494 PMID: 25159043

47. Kamolvit W, Sidjabat HE, Paterson DL. Molecular epidemiology and mechanisms of carbapenem resistance of *Acinetobacter* spp. in Asia and Oceania. Microb Drug Resist. 2015: 21:424–34. https://doi.org/10.1089/mdr.2014.0234 PMID: 25714653

48. Nigro SJ, Hall RM. Structure and context of Acinetobacter transposons carrying the oxa23 carbapenemase gene. J Antimicrob Chemother. 2016: 71:1135–47. https://doi.org/10.1093/jac/dkv440 PMID: 26755496

49. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect. 2006: 12:826–36. https://doi.org/10.1111/j.1469-0691.2006.01456.x PMID: 16882287

50. Ruiz M, Marti S, Fernandez-Cuenca F, Pascual A, Vila J. High prevalence of carbapenem-hydrolysing oxacillinases in epidemiologically related and unrelated *Acinetobacter baumannii* clinical isolates in Spain. Clin Microbiol Infect. 2007: 13:1192–8. https://doi.org/10.1111/j.1469-0691.2007.01825.x PMID: 17850347

51. Zong Z, Lü X, Valenzuela JK, Partridge SR, Iredell J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. Int J Antimicrob Agents. 2008: 31:50–4. https://doi.org/10.1016/j.ijantimicag.2007.08.019 PMID: 18077141

52. Anh NT, Nga TVT, Tuan HM, Tuan NS, Chau NVV, Baker S, et al. Molecular epidemiology and antimicrobial resistance phenotypes of *Acinetobacter baumannii* isolated from patients in three hospitals in southern Vietnam. J Med Microbiol. 2017: 66:46–53. https://doi.org/10.1099/jmm.0.000418 PMID: 28198682

53. Viana GF, Zago MCB, Moreira RRB, Zarpellon MN, Menegucci TC, Cardoso CL, et al. ISAba1/blaOXA-23: A serious obstacle to controlling the spread and treatment of *Acinetobacter baumannii* strains. Am J Infect Control. 2016: 44:593–5. https://doi.org/10.1016/j.ajic.2015.11.020 PMID: 26804302

54. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents. 2012: 39:105–14. https://doi.org/10.1016/j.ijantimicag.2011.10.004 PMID: 22113193

55. Opazo A, Domínguez M, Bello H, Amyes SG, González-Rocha G. OXA-type carbapenemases in *Acinetobacter baumannii* in South America. J Infect Dev Ctries. 2011: 6:311–6.

56. Catel-Ferreira M, Coadou G, Molle V, Mugnier P, Nordmann P, Siroy A, et al. Structure–function relationships of CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. J Antimicrob Chemother. 2011: 66:2053–6. https://doi.org/10.1093/jac/dkr267 PMID: 21705362

57. Coyne S, Courvalin P, Périchon B. Efflux-mediated antibiotic resistance in *Acinetobacter* spp. Antimicrob Agents Chemother. 2011: 55:947–53. https://doi.org/10.1128/AAC.01388-10 PMID: 21173183

58. Damier-Piolle L, Magnet S, Brémont S, Lambert T, Courvalin P. AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2008: 52:557–62. https://doi.org/10.1128/AAC.00732-07 PMID: 18086852

59. Gerson S, Nowak J, Zander E, Ertel J, Wen Y, Krut O, et al. Diversity of mutations in regulatory genes of resistance-nodulation-cell division efflux pumps in association with tigecycline resistance in *Acinetobacter baumannii*. J Antimicrob Chemother. 2018: 73:1501–1508. https://doi.org/10.1093/jac/dky083 PMID: 29554339

60. Zavascki AP, Carvalhaes CG, Picão RC, Gales AC. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: resistance mechanisms and implications for therapy. Expert Rev Anti Infect Ther. 2010: 8:71–93. https://doi.org/10.1586/eri.09.108 PMID: 20014903

61. Rahal JJ. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. Clin Infect Dis. 2006: 43:S95–S99. https://doi.org/10.1086/504486 PMID: 16894522

62. Casal M, Rodríguez F, Johnson B, Garduno E, Tubau F, de Lejarazu RO, et al. Influence of testing methodology on the tigecycline activity profile against presumably tigecycline-non-susceptible *Acinetobacter* spp. J Antimicrob Chemother. 2009: 64: 69–72. https://doi.org/10.1093/jac/dkp169 PMID: 19451133

63. Coelho-Souza T, Reis JN, Martins N, Martins IS, Menezes AO, Reis MGD, et al. Longitudinal surveillance for meningitis by Acinetobacter in a large urban setting in Brazil. Clin Microbiol Infect. 2013: 19: E241–4. https://doi.org/10.1111/1469-0691.12145 PMID: 23398654

64. Martins N, Picão RC, Adams-Sapper S, Riley LW, Moreira BM. Association of class 1 and 2 integrons with multidrug-resistant *Acinetobacter baumannii* international clones and Acinetobacter nosocomialis isolates. Antimicrob Agents Chemother. 2015: 59:698–701. https://doi.org/10.1128/AAC.02415-14 PMID: 25348522

65. Wang X, Zong Z, Lü X. Tn2008 is a major vehicle carrying blaOXA-23 in *Acinetobacter baumannii* from China. Diagn Microbiol Infect Dis. 2011: 69:218–22. https://doi.org/10.1016/j.diagmicrobio.2010.10.018 PMID: 21251570

66. Chen Y, Gao J, Zhang H, Ying C. Spread of the blaOXA–23-Containing Tn2008 in Carbapenem-Resistant *Acinetobacter baumannii* Isolates Grouped in CC92 from China. Front Microbiol. 2017: 8:163. https://doi.org/10.3389/fmicb.2017.00163 PMID: 28220115

67. Hamidian M, Hall RM. ISAba1 targets a specific position upstream of the intrinsic ampC gene of *Acinetobacter baumannii* leading to cephalosporin resistance. J Antimicrob Chemother. 2013: 68:2682–3. https://doi.org/10.1093/jac/dkt233 PMID: 23788477

68. Karah N, Dwibedi CK, Sjöström K, Edquist P, Johansson A, Wai SN, et al. Novel aminoglycoside resistance transposons and transposon-derived circular forms detected in carbapenem-resistant *Acinetobacter baumannii* clinical isolates. Antimicrob Agents Chemother. 2016: 60:1801–18. https://doi.org/10.1128/AAC.02143-15 PMID: 26824943

69. Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual Á. Plasmid-mediated quinolone resistance: an update. J Infect Chemother. 2011: 17:149–82. https://doi.org/10.1007/s10156-010-0120-2 PMID: 20886256

70. Jiang X, Yu T, Jiang X, Zhang W, Zhang L, Ma J. Emergence of plasmid-mediated quinolone resistance genes in clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Henan, China. Diagn Microbiol Infect Dis. 2014: 79:381–3. https://doi.org/10.1016/j.diagmicrobio.2014.03.025 PMID: 24805186

71. Araujo BF, Ferreira ML, de Campos PA, Royer S, da Fonseca Batistão DW, Dantas RCC, et al. Clinical and Molecular Epidemiology of Multidrug-Resistant *P. aeruginosa* carrying *aac(6')-Ib-cr*, *qnrS1* and bla_{SPM} Genes in Brazil. PLoS One. 2016: 11:e0155914. https://doi.org/10.1371/journal.pone.0155914 PMID: 27219003

72. Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, et al. Biology of *Acinetobacter baumannii*: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol. 2017: 7:55. https://doi.org/10.3389/fcimb.2017.00055 PMID: 28348979