International Journal of Antimicrobial Agents 53 (2019) 844–849

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Short Communication

Acinetobacter baumannii analysis by core genome multi-locus sequence typing in two hospitals in Bolivia: endemicity of international clone 7 isolates (CC25)

Mónica Cerezales[a,*], Kyriaki Xanthopoulou[b,c], Julia Wille[b,c], Zulema Bustamante[d], Harald Seifert[b,c], Lucía Gallego[a], Paul G. Higgins[b,c]

[a] Faculty of Medicine and Nursing, Immunology, Microbiology and Parasitology, University of the Basque Country UPV/EHU, Bilbao, Spain
[b] Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany
[c] German Centre for Infection Research, Partner Site Bonn-Cologne, Cologne, Germany
[d] Faculty of Biochemistry and Pharmacy, Universidad Mayor de San Simón, Cochabamba, Bolivia

ARTICLE INFO

Article history:
Received 3 September 2018
Accepted 20 March 2019

Keywords:
Acinetobacter baumannii
bla_{OXA-23}
International clone 7
Carbapenem-resistant
Tn*2008*

ABSTRACT

In total, 95 *Acinetobacter baumannii* isolates recovered from patients from two hospitals in Cochabamba, Bolivia were studied. The presence of class D and B β-lactamases was investigated using polymerase chain reaction, and antimicrobial susceptibility testing was performed by agar dilution and broth microdilution. The resistance rate to carbapenems was 53.7%. All carbapenem-resistant *A. baumannii* (CRAb, n=51) and four carbapenem-susceptible isolates were further analysed by whole-genome sequencing. The resulting genome assemblies were used to identify the acquired resistome, and core genome multi-locus sequence typing (cgMLST) was used to determine their molecular epidemiology. All but one of the CRAb isolates (n=50) belonged to international clone (IC) 7 and they clustered into five sequence types; on cgMLST, they were found to be separated by ≥40 alleles. All CRAb isolates carried bla_{OXA-23} on transposon Tn*2008*. Metallo-β-lactamases were not detected. These data show that dissemination of several IC7 *A. baumannii* clones harbouring the carbapenem resistance determinant bla_{OXA-23} is occurring in these two hospitals in Cochambamba.

© 2019 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

1. Introduction

Acinetobacter baumannii is an important nosocomial pathogen, and its prevalence in compromised patient groups and intensive care units (ICU) is a health challenge worldwide. It is responsible for a variety of infections including bloodstream infections, meningitis, ventilator-associated pneumonia, wound infections and urinary tract infections [1]. The World Health Organization published a priority list for research and development of new antimicrobials in 2017, and *A. baumannii* was set as the number one priority. The presence of different international clones (ICs), such as IC5 (Pan-American clone), IC4 and IC7, has been described in several Latin American countries [2–4]. Furthermore, in Latin America, there has been an increase in carbapenem-resistant *A. baumannii* (CRAb) isolates from 27% in 2006 to 76% in 2009, which mirrors the high prevalence of this pathogen [5]. Moreover, its unique ability to survive for long periods in dry environments contributes to its spread and persistance in the hospital setting [1].

The dissemination of these ICs is often associated with antimicrobial resistance, especially resistance to carbapenems. The spread of these ICs mirrors the increase in circulating carbapenemase encoding genes such as bla_{OXA-23}, which has been widely reported worldwide [5–7]. The most prevalent ICs in South America are IC5 ($CC79^{Pas}$) and IC7 ($CC25^{Pas}$), and this differs from the situation in North America and Europe where IC2 ($CC2^{Pas}$) is the predominant lineage. In South America and other regions, the number of CRAb isolates is also increasing [3,5–7].

As an additional problem, mobile genetic elements carrying antimicrobial resistance genes are also being disseminated among *A. baumannii* isolates. Carbapenemase encoding genes, particularly the most common in *A. baumannii*, the oxacillinases (OXAs), are often associated with insertion elements (IS) forming transposons [1,8] and with plasmids harbouring resistance genes [1].

The objective of this study was to determine the molecular epidemiology and to analyse the antimicrobial resistance rates of carbapenem-resistant *A. baumannii* isolates recovered from

* Corresponding author. Address: Faculty of Medicine and Nursing, Immunology, Microbiology and Parasitology, University of the Basque Country UPV/EHU, Barrio Sarriena s/n 48940, Bilbao, Spain. Tel.: +34946012772.
E-mail address: mcerezales001@ikasle.ehu.eus (M. Cerezales).

https://doi.org/10.1016/j.ijantimicag.2019.03.019
0924-8579/© 2019 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

patients in two Bolivian hospitals between September 2015 and December 2016.

2. Materials and methods

2.1. Bacterial isolates

In total, 95 *A. baumannii* isolates recovered between September 2015 and December 2016 from patients in two hospitals in close geographic proximity (Hospital Materno Infantil and Hospital Viedma) in the city of Cochabamba, Bolivia were included in this study. These two hospitals have a combined total of 408 beds and 21 ICU beds.

The isolates were initially identified as *Acinetobacter* spp. by biochemical methods in the hospital laboratories, and were confirmed as *A. baumannii* by *gyrB* multiplex polymerase chain reaction (PCR) [9] and the presence of $bla_{\text{OXA-51-like}}$ [10].

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the agar dilution method according to EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/). Minimum inhibitory concentrations (MICs) were determined for ciprofloxacin, gentamicin, imipenem, meropenem and tigecycline. For tigecycline, the EUCAST clinical MIC breakpoint for Enterobacteriaceae was used as no breakpoints are available for *A. baumannii*. The reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* subsp. *aureus* Rosenbach ATCC 29213 were used as control strains. Susceptibility testing was repeated three times for all isolates.

MICs for colistin in those carbapenem-resistant isolates with colistin MICs ≥2 mg/L by agar dilution were further tested by microbroth dilution using Micronaut-S MHK colistin plates (Merlin Diagnostika GmbH, Bornheim, Germany), with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 used as control strains.

2.3. Class D and B β-lactamases

A multiplex PCR was performed to detect the presence of genes encoding bla_{OXA} carbapenemases (51-like, 23-like, 58-like, 40-like, 143-like and 235-like) [11]. Additionally, two in-house multiplex PCRs were performed. The first PCR included the following genes: VIM, KPC, $bla_{\text{OXA-40-like}}$, NDM, $bla_{\text{OXA-48-like}}$ and $bla_{\text{OXA-23}}$. IMI, $bla_{\text{OXA-58-like}}$, GES, GIM, IMP and IS*Aba1*-$bla_{\text{OXA-51-like}}$ were screened by the second PCR [12].

2.4. DNA extraction and whole-genome sequencing

All CRAb isolates, CRAb (n=51) and a subset of carbapenem-susceptible (n=4) isolates were further investigated by whole-genome sequencing (WGS). Total DNA was prepared using the MagAttract HMW DNA kit (Qiagen, Hilden, Germany). Sequencing libraries were prepared using the Nextera XT library prep kit (Illumina GmbH, Munich, Germany) for a 250-bp paired-end sequencing run on an Illumina MiSeq sequencer. The FASTQ files containing paired reads were assembled de novo with the Velvet assembler using Ridom SeqSphere+ v.3.0 and SPAdes 3.9 (https://cge.cbs.dtu.dk/services/SPAdes/).

2.5. Molecular epidemiology and whole-genome sequencing analysis

A core genome multi-locus sequence typing (cgMLST) scheme was defined using Ridom SeqSphere+ v.3.0, with *A. baumannii* ACICU used as the reference genome. The resulting core genome of 2390 alleles was used to investigate the molecular epidemiology of the isolates [13]. A minimum spanning tree based on the core genome of 2390 alleles was generated using Ridom SeqSphere+, ignoring the missing values.

The assembled genomes were used to identify the acquired resistome using ResFinder 2.1 (https://cge.cbs.dtu.dk/services/ResFinder/). Sequence types were determined using the traditional seven loci MLST schemes of Oxford and Pasteur (https://pubmlst.org/abaumannii/). The $bla_{\text{OXA-51}}$ variant, sequence type (ST) as determined by the Pasteur scheme and cgMLST were also used to assign the isolates to an IC [13].

2.6. Determination of the gene location

S1 nuclease-pulsed field gel electrophoresis (S1-PFGE) and Southern blot hybridization were perfomed to determine the plasmid size and the plasmid/chromosomal location of $bla_{\text{OXA-23}}$ and *strA*, a gene conferring resistance to aminoglycosides, in a selection of isolates representing all the ICs and unique Oxford STs (n=10). Total bacterial DNA embedded in agarose plugs was digested with 50 U of S1 nuclease (Thermo Fisher Scientific, Waltham, MA, USA), incubated at 37°C for 45 min and separated using a CHEF-DR II system (Bio-Rad Laboratories, Hercules, CA, USA). The PFGE conditions were 17 h at 6 V/cm and 14°C. Initial and final pulses were conducted at 4 and 16 s, respectively.

DNA was transferred to a Hybond-N membrane by capillary transfer followed by hybridization with digoxigenin-labelled specific probes (Roche, Mannheim, Germany) for $bla_{\text{OXA-23-like}}$ and *strA*. Chromosomal location was shown by co-localization with the $bla_{\text{OXA-51-like}}$ probe. Signal detection was performed according to the manufacturer's instructions using CDP-Star ready-to-use chemiluminescent substrate (Roche) by autoradiography on an X-ray film.

2.7. Plasmid analysis

Plasmid analysis was performed by combining the acquired resistome with S1-PFGE and Southern blot hybridization with WGS data. Overlapping of putative plasmid contigs carrying resistance genes from assembled genomes was examined. Plasmid assemblies were further confirmed using PCR-based gap closure [14].

3. Results

3.1. Bacterial isolates: antimicrobial susceptibility and polymerase chain reaction experiments

All isolates were confirmed as *A. baumannii* by *gyrB* multiplex PCR and presence of the intrinsic $bla_{\text{OXA-51-like}}$ carbapenemase gene. The most prevalent source of the isolates was the respiratory tract (n=34; 35.8%), followed by wound secretions (n=17; 17.9%), ulcers (n=9, 9.5%) and urine culture (n=8; 8.4%). The rest of the isolates were recovered from diverse sources such as blood cultures, catheters, abscesses and exudates.

MICs for the tested antibiotics are summarized in Table 1, and resistance rates to ciprofloxacin, gentamicin, imipenem, meropenem and tigecycline are shown in Table 2. All of the isolates except one, MC96 ST1489$^{\text{Ox}}$-ST25$^{\text{Pas}}$ (MIC = 8 mg/L), were susceptible to colistin (MICs = 1–2 mg/L). MC96 was resistant to all the tested antimicrobial agents and is considered to be pan-drug resistant. The carbapenem resistance rate in Hospital Materno Infantil was higher than that in Hospital Viedma (66.7% vs. 48.7%, respectively).

By using multiplex PCR, the $bla_{\text{OXA-23-like}}$ gene was detected in the 51 CRAb isolates. No other acquired class D β-lactamases and no metallo-β-lactamases were detected in any of the isolates.

Table 1
Epidemiological data of the 55 sequenced isolates, minimum inhibitory concentrations of the tested antibiotics, results of S1 nuclease-pulsed field gel electrophoresis (S1-PFGE) and Southern blot hybridization, and the resistome.

			Isolate	Hospital	COL	IPM	MEM	CIP	TGC	GEN	S1-PFGE and Southern blot	Resistome
CRAb	IC7	$ST991_{pas}/ST1518_{ox}$	MC1	HMI	0.5 S	32 R	64 R	>128 R	16 R	32 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC2	HMI	1 S	32 R	32 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC3	HMI	1 S	32 R	32 R	128 R	4 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC5	HMI	1 S	32 R	32 R	128 R	4 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC6	HMI	1 S	32 R	32 R	64 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC8	HMI	1 S	32 R	64 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC12	HMI	1 S	32 R	32 R	>128 R	8 R	32 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC19	HMI	1 S	32 R	32 R	64 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC21	HMI	1 S	32 R	32 R	64 R	0.5 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC22	HMI	1 S	32 R	32 R	64 R	1 S	1 S	N.P.	*bla*$_{OXA-23}$ IS*Aba1-ampC*
			MC33	HV	0.5 S	32 R	32 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC35	HV	1 S	32 R	64 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC44	HV	0.25 S	32 R	64 R	>128 R	4 R	>128 R	N.P.	*bla*$_{OXA-23}$ IS*Aba1-ampC*
			MC50	HV	0.25 S	32 R	64 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC53	HV	1 S	32 R	32 R	32 R	0.5 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC60	HV	0.5 S	32 R	32 R	64 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC62	HV	0.25 S	32 R	32 R	32 R	0.5 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC105	HV	1 S	32 R	32 R	32 R	1 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
	IC7	$ST25_{pas}/ST1489_{ox}$	MC31	HV	1 S	32 R	32 R	128 R	8 R	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC32	HV	1 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC87	HV	1 S	32 R	64 R	128 R	8 R	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC89	HV	1 S	32 R	64 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC90	HV	1 S	32 R	64 R	64 R	4 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC91	HV	1 S	32 R	64 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC93	HV	1 S	32 R	64 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa* $_{64}$ *bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC94	HV	1 S	32 R	64 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC95	HV	1 S	32 R	64 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC96	HV	8 R	32 R	64 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC98	HV	1 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC100	HV	1 S	32 R	32 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC101	HV	1 S	32 R	32 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC102	HV	2 S	32 R	32 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC103	HV	1 S	32 R	64 R	64 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC104	HV	1 S	32 R	32 R	128 R	4 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
CRAb	IC7	$ST25_{pas}/ST1519_{ox}$	MC14	HMI	1 S	32 R	32 R	>128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC18	HMI	0.5 S	32 R	64 R	128 R	8 R	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC29	HV	0.5 S	32 R	64 R	>128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC34	HV	0.5 S	32 R	64 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC48	HV	0.5 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC59	HV	0.25 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC63	HV	0.25 S	32 R	32 R	>128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC64	HV	0.5 S	32 R	32 R	128 R	4 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC69	HV	1 S	32 R	32 R	>128 R	4 R	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC78	HV	1 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
	IC7	$ST25_{pas}/ST1529_{ox}$	MC39	HV	1 S	32 R	64 R	>128 R	8 R	4 S	N.P.	*bla*$_{OXA-23}$ IS*Aba1-ampC*
			MC51	HV	0.5 S	32 R	32 R	128 R	8 R	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC57	HV	0.5 S	32 R	32 R	128 R	4 R	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
	IC7	$ST25_{pas}/ST1528_{ox}$	MC27	HV	1 S	32 R	32 R	32 R	0.5 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC71	HV	1 S	32 R	64 R	64 R	0.5 S	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~180 Kb p#	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
			MC77	HV	1 S	32 R	32 R	32 R	0.5 S	>128 R	N.P.	*strA strB aac(3)-IIa bla*$_{OXA-23}$ IS*Aba1-ampC sul2 tet(B)*
	IC4	$ST15_{pas}/ST236_{ox}$	MC75	HV	1 S	32 R	64 R	128 R	2 S	>128 R	*bla*$_{OXA-23}$ chrom*/ *strA* ~150 Kb p#	*strA strB aac(3)-IIa aph(3')-VIa bla*$_{OXA-23}$ IS*Aba1-ampC bla*$_{TEM-1B}$ *sul2*
Non-CRAb	IC5	$ST79_{pas}/ST1520_{ox}$	MC23	HV	1 S	1 S	2 S	>128 R	4 R	>128 R	*strA* chrom*	*strA strB aadA1 aadB aph(3')-VIa* IS*Aba1-ampC bla*$_{TEM-1A}$ *sul2 floR dfrA1*
		$ST79_{pas}/ST233_{ox}$	MC17	HMI	1 S	1 S	2 S	>128 R	4 R	>128 R	N.P.	*strA strB aadA1 aadB aph(3')-VIa* IS*Aba1-ampC bla*$_{TEM-1A}$ *sul2 floR dfrA1*
			MC38	HV	1 S	0.5 S	1 S	>128 R	2 S	>128 R	N.P.	*strA strB aadA1 aadB aph(3')-VIa* IS*Aba1-ampC bla*$_{TEM-1A}$ *sul2 floR dfrA1*
	Sg^{β}	$ST267_{pas}/ST942_{ox}$	MC47	HV	1 S	0.5 S	2 S	128 R	0.5 S	>128 R	*strA* ~180 Kb p#	*strA strB aac(3)-IIa aph(3')-VIa ampC bla*$_{TEM-1B}$ *sul2 tet(B)*

COL, colistin; IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; TGC, tigecycline, GEN, gentamicin; S, susceptible; R, resistant. Sg^{β}, singleton; HV, Hospital Viedma; HMI, Hospital Materno Infantil. chrom*, chromosome; p#, plasmid; N.P., S1-PFGE+Southern blot not performed in these isolates.

Table 2
Minimum inhibitory concentrations (MICs) as determined by agar dilution for ciprofloxacin, gentamicin, imipenem, meropenem and tigecycline in all 95 isolates.

		Total (*n*=95)		CRAb (*n*=51)		Non-CRAb (*n*=44)	
		Susceptible *n* (%)	Resistant *n* (%)	Susceptible *n* (%)	Resistant *n* (%)	Susceptible *n* (%)	Resistant *n* (%)
Ciprofloxacin (*n*=95)		9 (9.47%)	86 (90.53%)	0 (0%)	51 (100%)	9 (20.45%)	35 (79.54%)
Colistin	BMD (*n*=39)	38 (97.44%)	1 (2.56%)	34 (66.67%)	1 (1.96%)	4 (9.10%)	0 (0%)
	Agar dilution (*n*=56)	56 (100%)	0 (0%)	16 (31.37%)	0 (0%)	40 (90.90%)	0 (0%)
Gentamicin (*n*=95)		12 (12.63%)	83 (87.37%)	2 (3.92%)	49 (96.08%)	10 (22.73%)	34 (77.27%)
Imipenem (*n*=95)		44 (46.32%)	51 (53.68%)	0 (0%)	51 (100%)	44 (100%)	0 (0%)
Meropenem (*n*=95)		44 (46.32%)	51 (53.68%)	0 (0%)	51 (100%)	44 (100%)	0 (0%)
Tigecycline (*n*=95)		37 (38.95%)	58 (61.05%)	17 (33.33%)	34 (66.67%)	20 (45.45%)	24 (54.54%)

CRAb, carbapenem-resistant *Acinetobacter baumannii*; BMD, broth microdilution method.
Colistin MICs were tested using the BMD in 39 isolates and the agar dilution method in 56 isolates.

Furthermore, IS*Aba1* was not detected upstream of the intrinsic $bla_{\text{OXA-51-like}}$ gene.

3.2. Molecular epidemiology and whole-genome sequencing analysis

Seven loci MLST revealed that the majority of the isolates [90.9% (*n*=50)] were ST25 or its single locus variant (SLV) ST991 according to the Pasteur scheme [clonal complex 25 (CC25)] which is associated with IC7. The majority of the isolates from Hospital Materno Infantil (77%) were IC7 ($ST991^{Pas}$), two were $ST25^{Pas}$ and one was IC5; the remaining $ST25^{Pas}$ isolates were found in Hospital Viedma. The IC7 isolates had $bla_{\text{OXA-64}}$, the characteristic $bla_{\text{OXA-51}}$ variant from this lineage. Furthermore, these isolates clustered with IC7 control strains using cgMLST (data not shown). These three pieces of evidence showed that IC7 was predominant among these *A. baumannii* isolates. Following the Oxford scheme, the 50 IC7 isolates were further delineated into five different groups belonging to CC110 [SLV: ST1489, ST1519, ST1529 and ST1518; double locus variant (DLV): ST1528].

The higher resolution of cgMLST showed that most isolates were not from the same clone (Fig. 1). There were five potential transmission clusters using a cut-off of 0–10 allelic differences. The other CC25 isolates differed by up to 110 alleles.

Five unrelated isolates differed from the IC7 group by over 1950 alleles. The three isolates carrying $bla_{\text{OXA-65}}$ were ST79 (IC5) according to the Pasteur scheme, and ST233 and DLV ST1520 according to the Oxford scheme, respectively, and clustered with the IC5 control strain by cgMLST. One isolate clustered with the IC4 control strains, carried $bla_{\text{OXA-51}}$, and was ST15 according to the Pasteur scheme and ST236 according to the Oxford scheme. One isolate was a singleton, carrying $bla_{\text{OXA-180}}$; this was $ST942^{Ox}$/$ST267^{Pas}$ and was not considered to represent one of the ICs.

WGS analysis confirmed that all the 51 CRAb isolates carried the $bla_{\text{OXA-23}}$ gene on *Tn2008*. Other genes conferring resistance to antibiotics such as aminoglycosides (*strA, n*=49; *strB, n*=49; *aac(3')-IIa, n*=49; *aac(3')-VIa, n*=5; *aadA1, n*=3; *aadB, n*=3), β-lactams (*amp*C, *n*=55; $bla_{\text{TEM-1A}}$, *n*=4; $bla_{\text{TEM-1B}}$, *n*=2), sulphonamides (*sul2, n*=52), tetracyclines (*tet(B), n*=48), trimethoprim (*dfrA, n*=3) and phenicols (*floR, n*=3) were also present. Additionally, the three isolates belonging to IC5, the IC4 isolate and the singleton carried the *aac(3')-VIa* gene located on a Tn*aphA6* transposon.

Results of the molecular epidemiology and acquired resistance genes of the isolates are shown in Table 1.

3.3. Location of resistance genes

Southern blot analysis revealed that $bla_{\text{OXA-23}}$ was located on the chromosome in all tested isolates (*n*=8). However, the *strA* gene was encoded on a ~180-Kb plasmid in all the IC7 isolates as well as in the singleton; the IC4 isolate carried the *strA* gene on a ~150-Kb plasmid. Genome assemblies and PCR-based gap closure revealed that *strA* was always linked to *strB, sul 2, aac(3)-IIa* and *tetB* in the IC7 isolates. No *tetB* gene was detected in the IC4 isolate. In contrast, Southern blot analysis revealed that *strA* was located on the chromosome in the IC5 isolates, and was associated with *strB, sul2* and *floR*. Additionally, the IC5 isolates harboured *aadB* on a ~6-Kb plasmid, already described and named 'pRAY' [15].

4. Discussion

IC7 isolates have been described previously in some Latin American countries, such as Paraguay and Argentina, but were usually sporadic isolates [2]. However, IC7 was the most prevalent group in studies performed in Bolivia or Uruguay [7], which is in concordance with the present findings. This study found only one carbapenem-resistant IC4 isolate and three carbapenem-susceptible IC5 isolates, although IC5 is the prevalent clonal lineage found in Latin America, the so-called 'Pan-American clone', followed by IC4 [6,7,16,17]. The prevalence of IC7 isolates suggests a change in the epidemiology of carbapenem-resistant *A. baumannii* isolates in Bolivia, particularly in the city of Cochabamba, when comparing these results with previous studies [17].

According to the Pan American Health Organization (PAHO) annual study, in Bolivia, 19% of *Acinetobacter* spp. were resistant to imipenem in 2010; in 2014, 51% of *A. baumannii* isolates were resistant to imipenem and 57% were resistant to meropenem [18,19]. This study found similar results to those obtained by PAHO in 2014, and the resistance rates were similar to those in Colombia, where the presence of ST229 (Oxford) isolates (with $bla_{\text{OXA-64}}$) and others that belong to IC7 have also been reported [20]. High rates of carbapenem resistance in Hospital Materno Infantil have been described in a previous study [21]. In addition, as can be seen in Table 2, the CRAb isolates present higher resistance rates to other antimicrobials, such as ciprofloxacin and gentamicin, compared with the non-CRAb isolates, thus complicating antimicrobial treatment options. When analysing the population within both hospitals, it can be seen that different clusters are associated with each of them; almost all the isolates from Hospital Materno Infantil were $ST991^{Pas}$, while $ST25^{Pas}$ isolates were mainly found in Hospital Viedma. Just two $ST25^{Pas}$ isolates were found in Hospital Materno Infantil. Some $ST991^{Pas}$ isolates were also isolated in Hospital Viedma (*n*=8), which may suggest that there is cross-transmission of $ST991^{Pas}$ from Hospital Materno Infantil to Hospital Viedma (Fig. 1).

Diverse $bla_{\text{OXA-51-like}}$ variants such as $bla_{\text{OXA-65}}$, $bla_{\text{OXA-64}}$, $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-66}}$ have been reported in Latin America, but until now, no $bla_{\text{OXA-180}}$ carbapenemase had been found [6]. Moreover, Sennati et al. described the presence of Tn*2008* in an ST25 (Pasteur) *A. baumannii* isolate in Bolivia with the intrinsic $bla_{\text{OXA-64}}$ [22]. In the Tn*2008* transposon, IS*Aba1* is not only serving as a

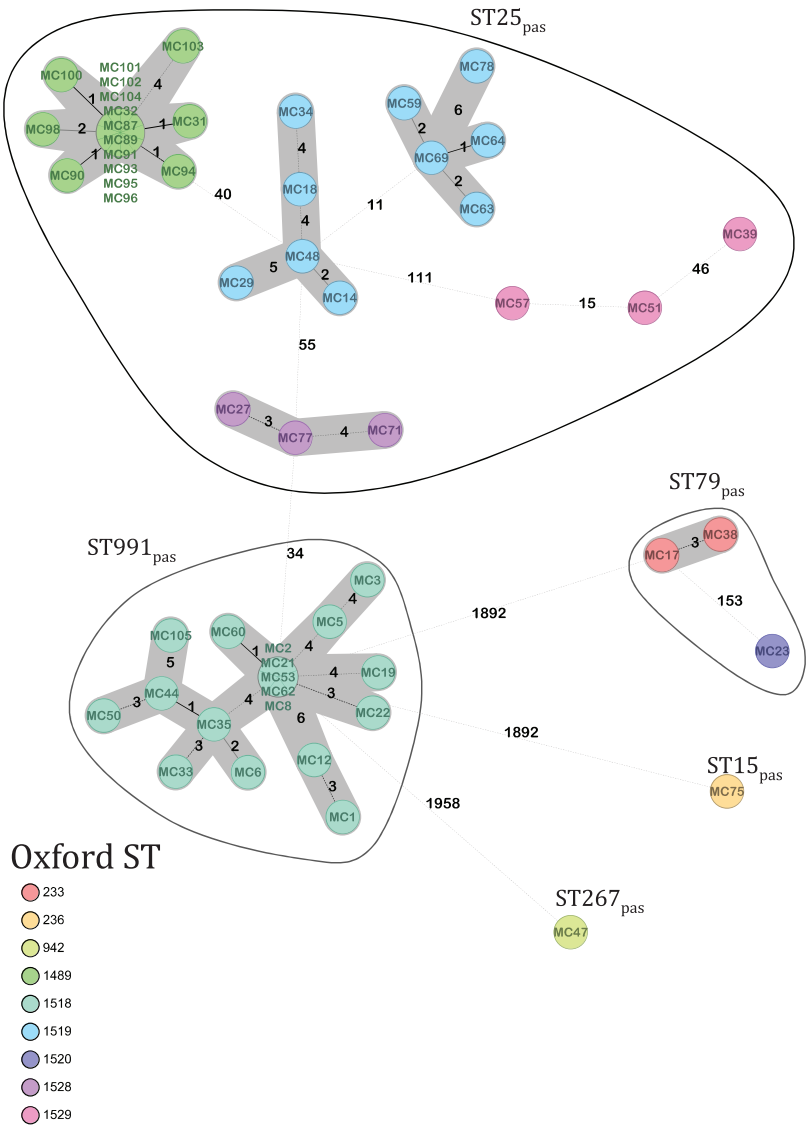

Fig. 1. Minimum spanning tree generated using Ridom SeqSphere+ for 55 samples, ignoring missing values. Distance based on columns from *Acinetobacter baumannii* core genome multi-locus sequence typing 2390 targets ACICU. Numbers between the nodes indicate the number of allelic differences. Shaded nodes represent transmission clusters. MC17, MC23, MC38 and MC47 are the carbapenem-susceptible *A. baumannii* isolates. There may be cross-transmission of ST991[Pas] between Hospital Materno Infantil and Hospital Viedma.

promoter for the carbapenemase encoding gene, but is also involved in mobilization of the gene [8]. This carbapenemase encoding vehicle has spread worldwide [8] and, in common with Sennati et al., the present study found it has been acquired by all the isolates belonging to ST25 (IC7) and the ST15 isolate (IC4). The increasing resistance rates to carbapenems were mediated by the mobilization of Tn*2008* among diverse ICs [8].

Furthermore, these isolates carried resistance genes encoded on different structures such as transposons or plasmids that can also spread and confer antimicrobial resistance to other groups of drugs such as aminoglycosides. In combination with carbapenem resistance, this further reduces the remaining therapeutic options [5].

Despite a number of published studies from South America [2,5,7,16,20], the epidemiology of *Acinetobacter* spp. is not well known for Bolivia. This study of CRAb isolates shed some light on the population dynamics of *A. baumannii* between these two Bolivian hospitals, and demonstrated the endemicity and dissemination of several strains of CC25 (IC7) within both hospitals. Evidence of cross-transmission is important in order to implement effective infection control strategies in the hospital setting. Finally, the high rates of antimicrobial resistance, especially to the carbapenems – mediated by the resistance determinant Tn*2008* and the presence of a pan-drug-resistant *A. baumannii* isolate – is of great concern due to the endemicity and the potential for epidemic spread in these hospitals.

Funding

This work was supported by the Basque Government and University of the Basque Country [Grupo Consolidado del Sistema Universitario Vasco (IT1097-16)/UPV/EHU GIC15/143].

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.03.019.

References

[1] Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538–82.

[2] Cieslinski JM, Arend L, Tuon FF, Silva EP, Ekermann RGS, Dalla-Costa LM, et al. Molecular epidemiology characterization of OXA-23 carbapenemase-producing *Acinetobacter baumannii* isolated from 8 Brazilian hospitals using repetitive sequence-based PCR. Diagn Microbiol Infect Dis 2013;77:337–40.

[3] Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233–8.

[4] Sahl JW, Del Franco M, Pournaras S, Colman RE, Karah N, Dijkshoorn L, et al. Phylogenetic and genomic diversity in isolates from the globally distributed *Acinetobacter baumannii* ST25 lineage. Sci Rep 2015;5:15188.

[5] 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 2014;42:1–17.

[6] Escandón-Vargas K, Reyes S, Gutiérrez S, Villegas MV. The epidemiology of carbapenemases in Latin America and the Caribbean. Exp Rev Anti Infect Ther 2017;15:277–97.

[7] Rodríguez CH, Balderrama Yarhui N, Nastro M, Nuñez Quezada T, Castro Cañarte G, Magne Ventura R, et al. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in South America. J Med Microbiol 2016;65:1088–91.

[8] Nigro SJ, Hall RM. Structure and context of *Acinetobacter* transposons carrying the *oxa23* carbapenemase gene. J Antimicrob Chemother 2016;71:1135–47.

[9] Higgins PG, Lehmann M, Wisplinghoff H, Seifert H. *gyrB* multiplex PCR to differentiate between *Acinetobacter calcoaceticus* and *Acinetobacter* genomic species 3. J Clin Microbiol 2010;48:4592–4.

[10] Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. J Clin Microbiol 2006;44:2974–6.

[11] Higgins PG, Pérez-Llarena FJ, Zander E, Fernández A, Bou G, Seifert H. OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2013;57:2121–6.

[12] Biniossek L, Gerson S, Xanthopoulou K, Zander E, Kaase M, Seifert H, et al. Novel multiplex PCR for detection of the most prevalent carbapenemase genes in Gram-negative bacteria within Germany. In: 68th Annual Conference of the German Society for Hygiene and Microbiology, DGHM; 2016.

[13] Higgins PG, Prior K, Harmsen D, Seifert H. Development and evaluation of a core genome multilocus typing scheme for whole-genome sequence-based typing of *Acinetobacter baumannii*. PLoS One 2017;12:e0179228.

[14] Villa L, Poirel L, Nordmann P, Carta C, Carattoli A. Complete sequencing of an IncH plasmid carrying the $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-15}}$ and *qnrB1* genes. J Antimicrob Chemother 2012;67:1645–50.

[15] Segal H, Elisha BG. Characterization of the *Acinetobacter* plasmid, pRAY, and the identification of regulatory sequences upstream of an *aadB* gene cassette on this plasmid. Plasmid 1999;42:60–6.

[16] Stietz MS, Ramírez MS, Vilacoba E, Merkier AK, Limansky AS, Centrón D, et al. *Acinetobacter baumannii* extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III. Infect Genet Evol 2013;14:294–301.

[17] Lopes BS, Gallego L, Amyes SGB. Multi-drug resistance profiles and the genetic features of *Acinetobacter baumannii* isolates from Bolivia. J Infect Dev Ctries 2013;7:323–8.

[18] Pan American Health Organization. Informe Anual de la Red Latinoamericana de Vigilancia de la Resistencia a los Antibióticos – 2010. San José, Costa Rica: PAHO; 2010.

[19] Pan American Health Organization Informe Anual de la Red de Monitoreo/Vigilancia de la Resistencia a los Antibióticos y de Infecciones Asociadas a la Atención de la Salud – 2014. Rev Patol Trop 2014;43:1–102.

[20] Saavedra SY, Nuñez JC, Pulido IY, Gonzalez EB, Valenzuela EM, Reguero MT, et al. Characterisation of carbapenem-resistant *Acinetobacter calcoaceticus–A. baumannii* complex isolates in a third-level hospital in Bogotá. Colombia. Int J Antimicrob Agents 2008;31:389–91.

[21] Cerezales M, Ocampo-Sosa AA, Álvarez Montes L, Díaz Ríos C, Bustamante Z, Santos J, et al. High prevalence of extensively drug-resistant *Acinetobacter baumannii* at a children hospital in Bolivia. Pediatr Infect Dis J 2018;37:1118–23.

[22] Sennati S, Villagran AL, Bartoloni A, Rossolini GM, Pallecchi L. OXA-23-producing ST25 *Acinetobacter baumannii*: first report in Bolivia. J Glob Antimicrob Resist 2016;4:70–1.