Infection, Genetics and Evolution 19 (2013) 127–133

Contents lists available at SciVerse ScienceDirect

Infection, Genetics and Evolution

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

Clonal complexes 104, 109 and 113 playing a major role in the dissemination of OXA-carbapenemase-producing *Acinetobacter baumannii* in Southeast Brazil

Eduardo Carneiro Clímaco [a], Milena Locci de Oliveira [a], André Pitondo-Silva [a], Murilo Gomes Oliveira [b], Micheli Medeiros [c,d], Nilton Lincopan [c,d], Ana Lúcia da Costa Darini [a,*]

[a] Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil
[b] Departamento de Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal de Juiz de Fora, Brazil
[c] Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil
[d] Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 21 April 2013
Received in revised form 15 June 2013
Accepted 22 June 2013
Available online 6 July 2013

Keywords:
Acinetobacter baumannii
Multilocus sequence typing
PFGE, OXA carbapenemases
Carbapenem resistance
Molecular epidemiology

ABSTRACT

Carbapenem resistance among *Acinetobacter baumannii* strains isolated from clinical settings in Brazil has increased dramatically in the last 10 years due to the emergence and dissemination of OXA-type carbapenemase encoding genes. This study aimed to characterize the presence of carbapenem-hydrolyzing class D β-lactamases (CHDL)-encoding genes and clonal complexes playing a major role in the dissemination of OXA-carbapenemase-producing *A. baumannii* in Southeast Brazil.

A total of 74 *A. baumannii* strains isolated from patients admitted to 4 hospitals in Southeast Brazil were analyzed. Molecular characterization of strains revealed that 67 strains carried bla_{OXA-23} (72%), $bla_{OXA-143}$ (25%) or both genes (3%). PFGE analysis identified 12 PFGE clusters, grouping 26 pulsotypes. Two PFGE clusters were predominant, comprising more than 66% of OXA-producing *A. baumannii* isolates. Among 23 representative strains characterized by MLST-UO (Multilocus Sequence Typing Scheme – University of Oxford, http://pubmlst.org/abaumannii/), 14 different STs were identified, of which six were confirmed as novel sequence types (designated as STs 402–407). Most of these isolates belonged to clonal complexes CC104,CC109 or CC113, whereas three STs were singletons (ST339, 403 and 407).

In conclusion, the presence of bla_{OXA-23}- and $bla_{OXA-143}$-like genes was not related to specific ST/CC, suggesting that the dissemination of OXA-carbapenemase-encoding genes may involve different STs, in which the spread of OXA-23-like is most likely due to mobile elements (i.e., plasmids). In this regard, CC104, CC109 and CC113 played a major role as predominant CDHL-carrying clones, instead of CC92, which was not identified.

1. Introduction

Among the species of the *Acinetobacter* genus, *Acinetobacter baumannii* is the most clinically relevant, as it is frequently isolated from hospitalized patients, especially causing outbreaks in intensive care units (ICUs) (Peleg et al., 2008). Despite of its low pathogenicity, *A. baumannii* is able to survive in adverse environments for long periods of time, becoming one of the most important opportunistic pathogens of nosocomial infections such as ventilator-associated pneumonia, bloodstream infections, postoperative infections, meningitis and urinary tract infection (Peleg et al., 2008). Invasive procedures, such as central venous catheter and ventilator use, recent major surgery, prior antimicrobial treatment, prolonged hospitalization and prior *A. baumannii* colonization have been identified as risk factors for facilitating *A. baumannii* infections (Jang et al., 2009). Furthermore, *A. baumannii* has emerged as an important pathogen in military treatment facilities (O'Shea, 2012).

In the last years, carbapenem resistance has been continuously reported among *A. baumannii* isolates, causing major therapeutic problems worldwide. Although efflux pumps over expression and porin loss appears to contribute to carbapenem resistance, the production of carbapenem-hydrolyzing class D β-lactamases (CHDL) is being increasingly reported globally, constituting the main mechanism of carbapenem resistance among *Acinetobacter*

* Corresponding author. Address: Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP. Av. do Café, s/n, Monte Alegre, Riberão Preto, SP CEP 14040-903, Brazil. Tel.: +55 16 3602 4290; fax: +55 16 3602 4725.
E-mail addresses: aldarini@fcfrp.usp.br, aldarini@usp.br (A.L. da Costa Darini).

1567-1348/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.meegid.2013.06.024

species (Livermore, 2009; Lee et al., 2011; Zarrilli et al., 2013). Currently, there are six subclasses of CDHL associated with *A. baumannii*: intrinsic chromosomal OXA-51-like enzymes and acquired OXA-23-, OXA-24-, OXA-58-, OXA-143- and OXA-235-like. In contrast with other subclasses, the carbapenemase activity of naturally occurring OXA-51-like enzymes is associated with IS*AbaI*, agenetic element that contains a strong promoter (Walther-Rasmussen and Høiby, 2006). Whilst the acquired enzymes OXA-23-, OXA-24-, OXA-51- and OXA-58-like have been disseminated worldwide, OXA-143-like has been found in *A. baumannii* strains from Brazil and OXA-235-like enzymes were recently identified in ten isolates from USA and Mexico (Walther-Rasmussen and Høiby, 2006; Higgins et al., 2009, 2013).

Despite the fact that the role of OXA enzymes is well established, molecular epidemiology studies of CHDL-producing *A. baumannii* are extremely useful to rapidly identify the emergence and dissemination of international MDR *A. baumannii* clones and to analyze their ancestor and evolutionary relationship (Irfan et al., 2011).

This study aimed to investigate the distribution of carbapenemase- and extended-spectrum β-lactamase-encoding genes among carbapenem-resistant *A. baumannii* isolates recovered between 2008 and 2010, from 4 hospitals in Southeast Brazil, determining their genetic relationship by PFGE analysis. In addition, MLST was used to verify the lineage of isolates with different susceptibility patterns and pulsotypes.

2. Materials and methods

2.1. Bacterial isolates

Between August 2008 and January 2010, 74 non-repetitive clinical *A. baumannii* isolates recovered from different clinical samples (intravenous catheter, tracheal secretion, blood, urine, peritoneal fluid, wound, spit) were collected in four hospitals in the Southeast region of Brazil. Whilst sixty-seven isolates were characterized as resistant to carbapenems (CRAB), seven carbapenem-susceptible *A. baumannii* (CSAB) isolates were included in the study for comparative evaluation. *A. baumannii* isolates were identified by means of the Vitek 2 automated instrument ID system (Vitek AMS; bioMerieux Vitek Systems, Hazelwood, MO, USA), conventional biochemical tests (Vaneechoutte et al., 2011) and by detection of the bla_{OXA-51}-type gene (Turton et al., 2006). Confirmation was performed by PCR and sequencing of internal and flanking regions of the *rpoB* gene (La Scola et al., 2006).

2.2. Antimicrobial susceptibility test

Antimicrobial susceptibility profiles were determined by disk diffusion according to CLSI guidelines (CLSI, 2012a,b). The antibiotics tested were gentamicin, tobramycin, amikacin, ticarcillin–clavulanate, piperacillin–tazobactam, meropenem, imipenem, ceftazidime, cefotaxime, cefepime, ciprofloxacin, levofloxacin, colistin, ampicillin–sulbactam, doxycicline, minocycline, tigecycline and polymyxin B. A breakpoint zone diameter of $\geqslant$16/$\leqslant$12 mm was used to define susceptibility/resistance to tigecycline (Jones et al., 2007). The polymyxin resistance phenotype was confirmed by Etest (bioMérieux, France).

2.3. Molecular detection of beta-lactamases-encoding genes

Genomic DNA was extracted as described elsewhere (Pitcher et al., 1989) and multiplex polymerase chain reactions were performed to investigate the presence of CHDL-encoding genes (bla_{OXA-23}-, bla_{OXA-24}-, bla_{OXA-51}-, bla_{OXA-58}- and $bla_{OXA-143}$-like) using specific primers, as previously described (Woodford et al., 2006; Higgins et al., 2010). Additional screening of class B and A carbapenemase- and extended-spectrum beta-lactamase (ESBL)-encoding genes was performed by conventional PCR. Specific primers, described elsewhere (Saladin et al., 2002; Pitout et al., 2004; Rasheed et al., 1997), are quoted in Table 1. Amplifications were carried out in a total volume of 25 μL, with 60 ng of template DNA, 24 pmol of primers (Invitrogen, Carlsbad, California), 5 μL 10× buffer, 2 mmol/L $MgSO_4$ (Invitrogen), 0.5 μmol of each deoxynucleoside triphosphate (Eppendorf, Westbury, New York), and 0.625 U Taq High Fidelity DNA polymerase (Invitrogen). PCR reactions were performed in a Mastercycler Gradient (Eppendorf), using as cycling program an initial cycle of 5 min at 95 °C; followed by 30 cycles of 1 min at 95 °C, 1 min at annealing temperature (Table 1), 1 min at 72 °C; and a final extension cycle of 10 min at 72 °C. The PCR products were analyzed under 1% agarose gel electrophoresis and visualized by UV light after staining the gel with ethidium bromide.

2.4. Pulsed-field gel electrophoresis

Genomic DNA macrorestriction profiles of *A. baumannii* strains were determined by pulsed-field gel electrophoresis (PFGE) after DNA extraction and digestion using *SmaI* (Invitrogen, Paisley, UK), as described elsewhere (Tenover et al., 1995). DNA fragments were separated by electrophoresis in 1% ultra-pure agarose gels (Invitrogen) and 0.5× Tris–borate–EDTA buffer, at 14 °C, using the CHEFF III apparatus (Bio-Rad Laboratories, Hercules, California) at 6 V/cm^2 with switch times ranging from 5 s to 20 s at an angle of 120°, for 19 h. A standard molecular weight ladder (Lambda Ladder 50–1000 kb PFG Marker; New England Biolabs) was included twice in each gel to compare the fingerprints on different gels. Processed gels were stained with ethidiumbromide, and DNA banding pattern images were documented after transillumination with UV light in AlphaImage gel documentation system (Alpha Innotech, San Leandro, CA).

Bionumerics 5.01 software (Applied Maths, Ghent, Belgium) was used for dendrogram construction by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, based on Dice's similarity coefficient. Pulsotypes were defined as per the criteria described by Tenover (Tenover et al., 1995), whereas isolates were considered to belong to the same PFGE cluster if their Dice similarity index was $\geqslant$84%.

2.5. Multi locus sequence typing (MLST)

Twenty-three selected *A. baumannii* isolates showing different resistance patterns (phenotype/genotype) and representative of the major PFGE clusterss were subjected to MLST analysis (Bartual et al., 2005), using specific primers and conditions described in the *A. baumannii* MLST database of the University of Oxford (http://pubmlst.org/abaumannii). The amplicons were purified using GFX PCR (GE Healthcare, Chalfont St Giles, Buckinghamshire, UK) and sequenced using primers (forward and reverse) described in the database website (http://pubmlst.org/abaumannii), with the MegaBace™ 1000 DNA Sequencing System (GE Healthcare). The sequences obtained were analyzedusing the ChromasPro software version 1.5 (Technelysium Pty. Ltd., South Brisbane, Australia).

Sequence types (STs) were assigned using the MLST database and new MLST alleles and STs were assigned by the curators of the database. Finally, molecular epidemiology was established using the eBURST software (http://eburst.mlst.net/).

Table 1
Primers used in the study for PCR amplification of resistance genes.

Primer	Sequence (5′–3′)	Annealing Temperature (°C)	amplified fragment/length (pb)	Reference
OXA-51-F	TAATGCTTTGATCGGCCTTG	52	$bla_{\text{OXA-51-like}}$/353	Woodford et al., 2006
OXA-51-R	TGGATTGCACTTCATCTTGG	52		Woodford et al., 2006
OXA-23-F	GATCGGATTGGAGAACCAGA	52	$bla_{\text{OXA-23-like}}$/501	Woodford et al., 2006
OXA-23-R	ATTTCTGACCGCATTTCCAT	52		Woodford et al., 2006
OXA-40-F	GGTTAGTTGGCCCCCTTAAA	52	$bla_{\text{OXA-40-like}}$/246	Woodford et al., 2006
OXA-40-R	AGTTGAGCGAAAAGGGGATT	52		Woodford et al., 2006
OXA-58-F	AAGTATTGGGGCTTGTGCTG	52	$bla_{\text{OXA-58-like}}$/599	Woodford et al., 2006
OXA-58-R	CCCCTCTGCGCTCTACATAC	52		Woodford et al., 2006
OXA-143-F	TGGCACTTTCAGCAGTTCCT	52	$bla_{\text{OXA-143}}$/149	Higgins et al., 2010
OXA-143-R	TAATCTTGAGGGGGCCAACC	52		Higgins et al., 2010
IMP-P1	CGAGAAGCTTGAAGAAGGTGTT	60	$bla_{\text{IMP-2,-8,-13,-19,-20,-24}}$/512	This work
IMP-P2	CGGACTTTGGCCAAGCTTCTA	60		This work
IMP-P3	GAAGTTAACGGGTGGGGCGTT	60	$bla_{\text{IMP-1,-3,-4,-6,-10}}$/578	This work
IMP-P4	CCTTTAACCGCCTGCTCTAATG	60		This work
IMP-P5	GACAGTACGGSTGGAATAGAG	59	$bla_{\text{IMP-12, -14, -18}}$/407	This work
IMP-P6	CCTTTAACAGCCTGCTCCCA	59		This work
IMP-P7	GTAGCATTACTGCCGCAGGA	59	$bla_{\text{IMP-11,-16,-21,-22}}$/643	This work
IMP-P8	GTAAGCTTCAAGAGCGACGCA	59		This work
IMP-P9	CCTAAACACGGCTTGGTGGTT	59	$bla_{\text{IMP-5,-7,-9,-15}}$/349	This work
IMP-P10	GCCAAACCACTACGTTATCTGG	59		This work
VIM-P1	GTTATTGGTCTATTTGACCGCG	58	$bla_{\text{VIM-2,-3,-6,-8,-9,-10,-11,-18}}$/781	This work
VIM-P2	CTACTCAACGACTGAGCGATTT	58		This work
VIM-P3	GGTCTACATGACCGCGTCTGT	61	$bla_{\text{VIM-1,-4 -5,-14}}$/775	This work
VIM-P4	CTACTCGGCGACTGAGCGATT	61		This work
VIM-P5	CGATGGYGTTTGGTCGCATAT	58	$bla_{\text{VIM-7,-12,-13}}$/390	This work
VIM-P6	GAATGCGCAGCACCAGGATA	58		This work
SPM-F	GAGAGCCCTGCTTGGATTCAT	59	$bla_{\text{SPM-1}}$ / 811	This work
SPM-R	CAGTCTCATTTCGCCAACGG	59		This work
KPC-F	GTATCGCCGTCTAGTTCTGCTG	61	bla_{KPC} / 871	This work
KPC-R	GTTGACGCCCAATCCCTCGA	61		This work
MA1	SCSATGTGCAGYACCAGTAA	57	$bla_{\text{CTX-M-1,-2,-9}}$ groups/544	Saladin et al., 2002
MA2	CCGCRATATGRTTGGTGGTG	57		Saladin et al., 2002
CTXM1 f3	GACGATGTCACTGGCTGAGC	53	$bla_{\text{CTX-M-1}}$ group / 499	Pitout et al., 2004
CTXM1 r2	AGCCGCCGACGCTAATACA	53		Pitout et al., 2004
M2f	ATGATGACTCAGAGCATTCG	55	$bla_{\text{CTX-M-2}}$ group/866	Saladin et al., 2002
M2r	TGGGTTACGATTTTCGCCGC	55		Saladin et al., 2002
M9f	ATGGTGACAAAGAGAGTGCA	55	$bla_{\text{CTX-M-9}}$ group/870	Saladin et al., 2002
M9r	CCCTTCGGCGATGATTCTC	55		Saladin et al., 2002
M8f	CTGGAGAAAAGCAGCGGGGG	51	$bla_{\text{CTX-M-8,-25}}$ groups/581	This work
M8r	ACCCACGATGTGGGTAGCCC	51		This work
TEM f	ATGAGTATTCAACATTTCCG	58	bla_{TEM}	Rasheed et al., 1997
TEM r	CTGACAGTTACCAATGCTTA	58		Rasheed et al., 1997
SHV f	GGGTTATTCTTATTTGTCGC	56	bla_{SHV}	Rasheed et al., 1997
SHV r	TTAGCGTTGCCAGTGCTC	56		Rasheed et al., 1997

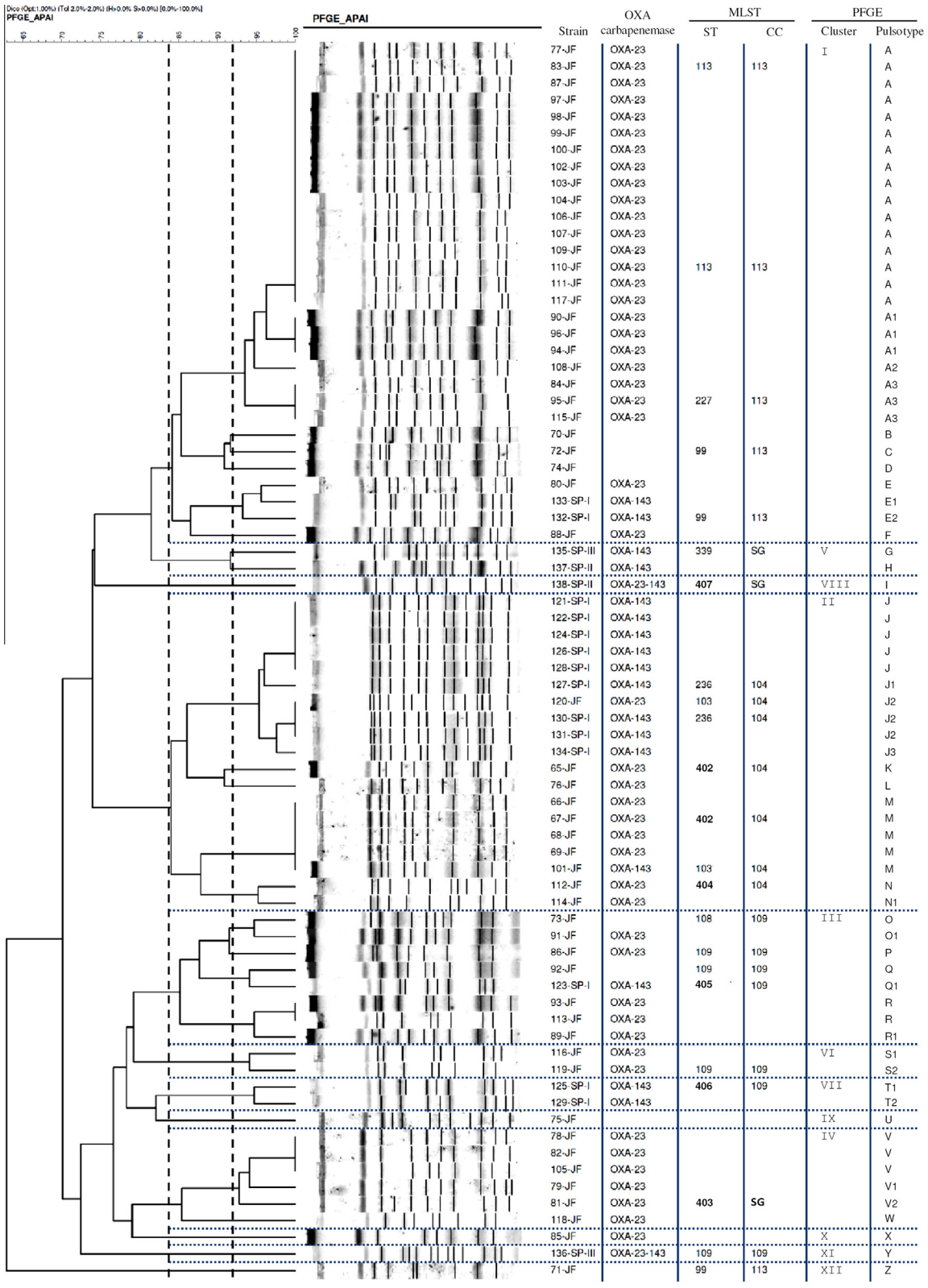
3. Results

All 67 CRAB isolates displayed resistance to cefotaxime, ceftazidime, cefepime, piperacillin–tazobactam and ciprofloxacin. Furthermore, non-susceptibility to ampicillin–sulbactam, gentamicin and levofloxacin occurred in 54%, 60%, and 84% of isolates, respectively. On one hand, CRAB isolates showed high susceptibility to colistin (100%), doxycicline (100%), polymyxin (90%) and tigecycline (90%). On the other hand, all 7 CSAB isolates were resistant to cefotaxime, ceftazidime, cefepime, ciprofloxacin, of which four isolates displayed non-susceptibility to gentamicin and levofloxacin, and other two *A. baumannii* isolates were non-susceptible to tigecycline and piperacillin–tazobactam.

Although genes encoding OXA-51 were detected in all CRAB/CSAB isolates included in the study, acquired CHDL-encoding genes were only identified among CRAB isolates. In this regard, the presence of $bla_{\text{OXA-23}}$- and $bla_{\text{OXA-143}}$-like genes was confirmed in 71.6% and 25.4% of CRAB, respectively, whereas both genes were identified in 3% of CRAB isolates. Genes encoding for class A or B carbapenemases, or ESBLs, were not found.

Based on Tenover's criteria (Tenover et al., 1995), PFGE analysis, the *A. baumannii* isolates were classified into 26 pulsotypes (named A–Z, in Fig. 1) and these subtypes were grouped into 12 different PFGE clusters (named I–XII, in Fig. 1). Two PFGE clusters (I and II) were predominant, grouping 30 and 19 isolates, respectively, whereas the other ten PFGE clusters contained up to 8 *A. baumannii* isolates each (Fig. 1). Clusters I and II grouped more 66% of the OXA-producing isolates and pulsotype A was predominant (23 isolates) (Fig. 1). The majority of OXA-23-like producing *A. baumannii* strains, belonging to pulsotype A, were recovered from the hospital JF, whereas OXA-143 positive strains, belonging to pulsotypes E, J, Q and T, were more prevalent in the hospital SP-I. Five out of the seven CSAB isolates were grouped into PFGE clusters with CRAB isolates (3 into PFGE cluster I, and 2 into PFGE cluster III). The other 2 CSBA isolates were unique in their PFGE clusters (IX and XII) (Fig. 1).

MLST analysis of the 23 *A. baumannii* isolates with different resistance patterns and representative of the major PFGE clusters revealed fourteen different STs including the previously described STs 99, 103, 108, 109, 113, 227, 236, 339; and the novel STs 402, 403, 404, 405, 406 and 407 (Fig. 1). Subsequent eBURST analysis showed that typed isolates from the representative PFGE clusters belonged to clonal complexes CC104 (ST103, 236, 402 and 404), CC109 (ST108, 109, 405 and 406) and CC113 (ST99, 113 and 227). Three STs described in this study were singletons (ST339, 403 and 407) (Fig. 2). Among the four CSAB isolates investigated by MLST, two were typed as ST99 (CC113), one as ST108 (CC109) and one as ST109 (CC109).



Strain	OXA carbapenemase	MLST ST	MLST CC	PFGE Cluster	PFGE Pulsotype
77-JF	OXA-23			I	A
83-JF	OXA-23	113	113		A
87-JF	OXA-23				A
97-JF	OXA-23				A
98-JF	OXA-23				A
99-JF	OXA-23				A
100-JF	OXA-23				A
102-JF	OXA-23				A
103-JF	OXA-23				A
104-JF	OXA-23				A
106-JF	OXA-23				A
107-JF	OXA-23				A
109-JF	OXA-23				A
110-JF	OXA-23	113	113		A
111-JF	OXA-23				A
117-JF	OXA-23				A
90-JF	OXA-23				A1
96-JF	OXA-23				A1
94-JF	OXA-23				A1
108-JF	OXA-23				A2
84-JF	OXA-23				A3
95-JF	OXA-23	227	113		A3
115-JF	OXA-23				A3
70-JF					B
72-JF		99	113		C
74-JF					D
80-JF	OXA-23				E
133-SP-I	OXA-143				E1
132-SP-I	OXA-143	99	113		E2
88-JF	OXA-23				F
135-SP-III	OXA-143	339	SG	V	G
137-SP-II	OXA-143				H
138-SP-II	OXA-23-143	407	SG	VIII	I
121-SP-I	OXA-143			II	J
122-SP-I	OXA-143				J
124-SP-I	OXA-143				J
126-SP-I	OXA-143				J
128-SP-I	OXA-143				J
127-SP-I	OXA-143	236	104		J1
120-JF	OXA-23	103	104		J2
130-SP-I	OXA-143	236	104		J2
131-SP-I	OXA-143				J2
134-SP-I	OXA-143				J3
65-JF	OXA-23	402	104		K
76-JF	OXA-23				L
66-JF	OXA-23				M
67-JF	OXA-23	402	104		M
68-JF	OXA-23				M
69-JF	OXA-23				M
101-JF	OXA-143	103	104		M
112-JF	OXA-23	404	104		N
114-JF	OXA-23				N1
73-JF		108	109	III	O
91-JF	OXA-23				O1
86-JF	OXA-23	109	109		P
92-JF		109	109		Q
123-SP-I	OXA-143	405	109		Q1
93-JF	OXA-23				R
113-JF	OXA-23				R
89-JF	OXA-23				R1
116-JF	OXA-23			VI	S1
119-JF	OXA-23	109	109		S2
125-SP-I	OXA-143	406	109	VII	T1
129-SP-I	OXA-143				T2
75-JF				IX	U
78-JF	OXA-23			IV	V
82-JF	OXA-23				V
105-JF	OXA-23				V
79-JF	OXA-23				V1
81-JF	OXA-23	403	SG		V2
118-JF	OXA-23				W
85-JF	OXA-23			X	X
136-SP-III	OXA-23-143	109	109	XI	Y
71-JF		99	113	XII	Z

Fig. 1. Dendrogram of genomic similarity representative of PFGE profiles presented by isolates of *Acinetobacter baumannii* using the UPGMA (Unweighted Pair Group Method with Arithmetic) method based on Dice's similarity. Dotted lines define the cutoff for clonal groups (84%) and for pulsotypes (92%). SG represents singleton STs. Hospitals (JF, SP-I, SP-II and SP-III) are indicated as following the strain identification.

MLST analysis of the 23 isolates showed that those grouped in CC113 were mainly located in cluster I. In contrast, isolates located in PFGE cluster II belonged to CC104. Isolates grouped in CC109 (International Clone I) showed pulsotypes located in four different PFGE clusters (III, VI, VII and XI). These results showed that PFGE typing was complementary to MLST to define isolates belonging to the international clone I. Even though OXA-23-like and OXA-143-like producing isolates were grouped in the three clonal

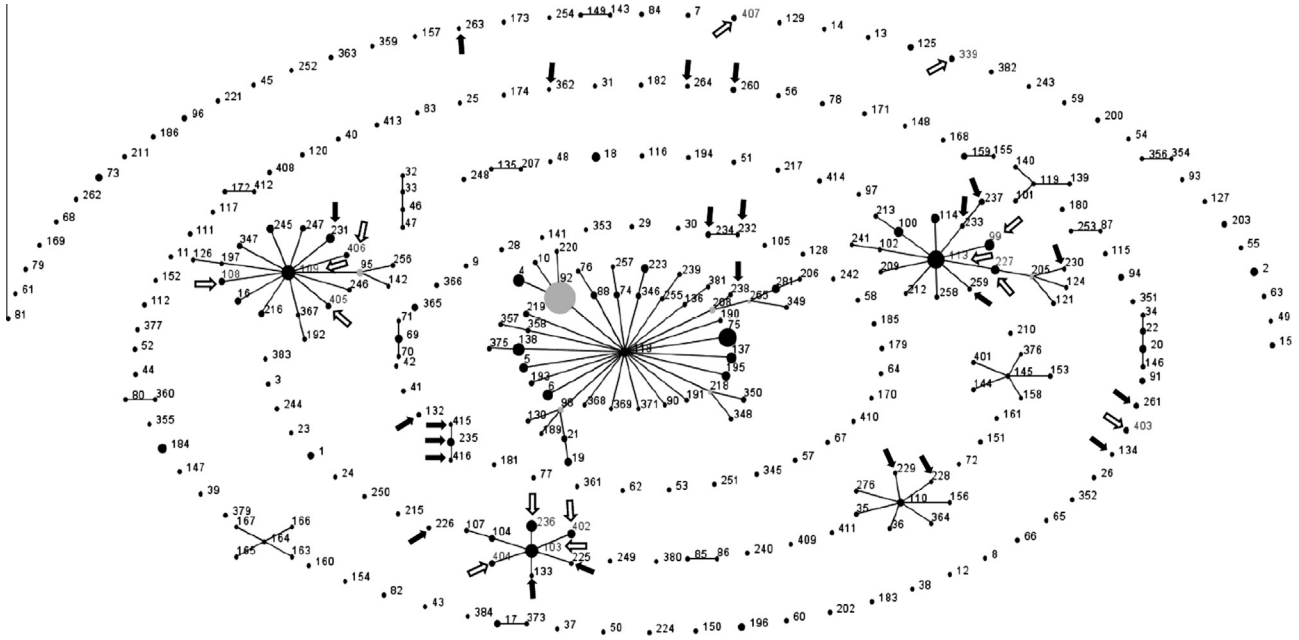


Fig. 2. eBURST diagram showing the analyzed sequence types (STs) of the Brazilian strains and all STs assigned for the entire public *A. baumannii* multilocus sequence type database (http://pubmlst.org/abaumannii/). Each ST is represented by a dot in proportional size to the number of *A. baumannii* involved, considering all strains in the database. STs related to *A. baumannii* strains from Brazil are indicated by arrows, of which white arrows indicate the STs related to the strains of this study. The main clonal complexes (CCs) are named next to them.

complexes found, ST and pulsotype distinguished the isolates harboring encoding genes of each enzyme in several cases.

Currently, the MLST database (http://pubmlst.org/abaumannii) includes 38 different STs of *A. baumannii* isolated from Brazil (Fig. 2). According to eBURST analysis, most CRAB strains isolated in the Southeast region of Brazil comprised isolates of different STs, clonally related and grouped into the clonal complexes CC104, CC109 and CC113, which included mainly single-locus variants (SLVs) or less commonly, double- (DLVs), or triple-locus variants (TLVs) (Fig. 2). Among CRAB isolates carrying acquired CHDL-encoding genes, three strains were characterized as singletons and six novel STs were identified.

4. Discussion

Carbapenem-resistant non-fermenter bacilli (mainly, *Pseudomonas aeruginosa* and *A. baumannii*) have become increasingly isolated in Brazil. In this regard, resistance to carbapenem among *A. baumannii* isolates has been mostly related to the production of CHDL (Dalla-Costa et al., 2003; Antonio et al., 2011; Mostachio et al., 2012; Martins et al., 2012), which was confirmed in this study, as bla_{OXA-23}- and $bla_{OXA-143}$-like were the most prevalent CHDL-encoding genes identified.

Worldwide dissemination of the bla_{OXA-23} carbapenemase gene has been associated with specific clones, mainly the international CC92, which is the largest and most widespread clonal complex in the *A. baumannii* MLST database, currently comprising 207 strains and 46 different STs. OXA-23-producing *A. baumannii* strains belonging to CC92 have been identified in many countries around the world, including Australia, United States of America (USA), China, Italy, France, Tahiti, Vietnam, New Caledonia, Thailand and South Africa (Adams-Haduch et al., 2011; Ansaldi et al., 2011; Fu et al., 2010; Lee et al., 2012; Nigro and Hall, 2012; Mugnier et al., 2010; Runnegar et al., 2010). Moreover, *A. baumannii* isolates carrying*ISAbaI-*bla_{OXA-51}-, bla_{OXA-58}-, or bla_{OXA-40}-like genes, belonging to the CC92, have been reported in Japan, Italy, and USA, respectively (Adams-Haduch et al., 2011; Ansaldi et al., 2011; Endo et al., 2012).

In Latin American countries, MLST allelic profiles have not been related to the worldwide-predominant ST92/OXA-23 *A. baumannii* (Grosso et al., 2011; Martins et al., 2013; Stietz et al., 2013) and, indeed, this phenomenon was also observed in this study, in which none of the twenty-three representative strains analyzed by MLST belonged to CC92. In contrast, CRAB carrying bla_{OXA-23}- and/or $bla_{OXA-143}$-like genes belonged mainly to CC104, CC109 and CC113. Since the acquired CHDL-encoding genes were not related to specific ST, it suggests that dissemination of these genes in Brazil should be associated with plasmid-mediated mobilization. This hypothesis is supported by Mugnier and co-workers (2010) which showed CRAB isolates not belonging to CC92 harboredbla_{OXA-23} gene located in similar plasmids (Mugnier et al., 2010).

In this respect, most OXA-23 producing *A. baumannii* strains isolated in Latin America have been commonly reported as belonging to CC113 (Grosso et al., 2011; Martins et al., 2013; Ramírez et al., 2013; Stietz et al., 2013). On the other hand, although OXA-23-producing *A. baumannii* isolates belonging to the international CC109 have been previously identified in Brazil, only the presence of ST231 has been reported so far (Martins et al., 2012). Thus, this study shows that other STs of *A. baumannii*, belonging to the CC109, have acquired CHDL-encoding genes, including isolates confirmed as novel sequence types, designated as STs 405 and 406, which have not been described elsewhere. Curiously, CRAB isolates of ST405 and ST406 harbored the novel$bla_{OXA-143}$ gene, whereas the one ST109 strain harbored both bla_{OXA-23}- and $bla_{OXA-143}$-like genes. Furthermore, two ST109 strains carried the bla_{OXA-23}-like gene, an association that has been previously identified in CRAB strains isolated in Europe, Africa and Persian Gulf countries (Ansaldi et al., 2011; Mugnier et al., 2010).

Regarding CC104, this clonal complex currently consists of eight STs comprising 24 strains, which have been mainly identified in Brazil and Argentina, and sporadically in Europe (Norway, Portugal,

Czech Republic, Netherlands, Turkey, Spain and Greece) (Karah et al., 2012; Martins et al., 2013; Ramírez et al., 2013). In line with previous reports, our results confirm the predominance of this clonal complex among CRAB isolates from Southeast Brazil (Martins et al., 2013), suggesting local endemicity associated with the acquisition of CHDL-encoding genes. Indeed, unlike CC109 and CC113, CC104 comprised only CRAB isolates, carrying bla_{OXA-23}- or $bla_{OXA-143}$-like genes of four different STs, of which two are reported as novel STs. In this regard, isolates of the new STs 402and 404 carried bla_{OXA-23}-like genes, whereas genes encoding OXA-143-like enzymes were found in CRAB isolates of ST236 and ST103.

Finally, this study confirms PFGE and MLST to be complementary tools in clonality studies. In this regard, it was possible to observe a good correlation between these two techniques, since most *A. baumannii* strains grouped into PFGE clusters I, II and III, were correlated to the MLST-defined CC113, CC104 and CC109, respectively.

5. Conclusion

In conclusion, the present study provides new data regarding the low frequency of the international CC92 (clone II) among carbapenem non-susceptible *A. baumannii* isolates in South America, highlighting the importance of the clonal complexes CC104, CC109 and CC113 in Southeast Brazil. In addition, the presence of bla_{OXA-23}- and $bla_{OXA-143}$-like genes was not related to specific ST/CC, suggesting that the spread of OXA-23-like is most likely due to mobile elements (i.e., plasmids). Consequently, the great adaptive potential of *A. baumannii* strains, hereby evidenced by the large amount of new STs found, may be one of the major factors contributing to the dissemination of OXA-producing *A. baumannii* in Brazil.

Acknowledgements

This study was financially supported by the grant #2012/08210-1, São Paulo Research Foundation (FAPESP). We are grateful to Dr. W. de Araujo da Silva Junior and A. Aparecida Marques for DNA sequencing and to J.C. Ferreira for the technical assistance provided.

References

Adams-Haduch, J.M., Onuoha, E.O., Bogdanovich, T., Tian, G.B., Marschall, J., Urban, C.M., Spellberg, B.J., Rhee, D., Halstead, D.C., Pasculle, A.W., Doi, Y., 2011. Molecular epidemiology of carbapenem-nonsusceptible Acinetobacter baumannii in the United States. J. Clin. Microbiol. 49, 3849–3854.

Ansaldi, F., Canepa, P., Bassetti, M., Zancolli, M., Molinari, M.P., Talamini, A., Ginocchio, F., Durando, P., Mussap, M., Orengo, G., Viscoli, C., Icardi, G., 2011. Sequential outbreaks of multidrug-resistant *Acinetobacter baumannii* in intensive care units of a tertiary referral hospital in Italy: combined molecular approach for epidemiological investigation. J. Hosp. Infect. 79, 134–140.

Antonio, C.S., Neves, P.R., Medeiros, M., Mamizuka, E.M., Elmor de Araújo, M.R., Lincopan, N., 2011. High prevalence of carbapenem-resistant *Acinetobacter baumannii* carrying the $bla_{OXA-143}$ gene in Brazilian hospitals. Antimicrob. Agents Chemother. 55, 1322–1323.

Bartual, S.G., Seifert, H., Hippler, C., Luzon, M.A., Wisplinghoff, H., Rodríguez-Valera, F., 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J. Clin. Microbiol. 43, 4382–4390.

CLSI, 2012a. Performance standards for antimicrobial disk susceptibility tests; approved standard – eleventh edition. Clinical and Laboratory Standards Institute 11, M02–A11.

CLSI, 2012b. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. Clinical and Laboratory Standards Institute 21, M100-S22.

Dalla-Costa, L.M., Coelho, J.M., Souza, H.A., Castro, M.E., Stier, C.J., Bragagnolo, K.L., Rea-Neto, A., Penteado-Filho, S.R., Livermore, D.M., Woodford, N., 2003. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J. Clin. Microbiol. 41, 3403–3406.

Endo, S., Yano, H., Hirakata, Y., Arai, K., Kanamori, H., Ogawa, M., Shimojima, M., Ishibashi, N., Aoyagi, T., Hatta, M., Yamada, M., Tokuda, K., Kitagawa, M., Kunishima, H., Kaku, M., 2012. Molecular epidemiology of carbapenem-non-susceptible *Acinetobacter baumannii* in Japan. J. Antimicrob. Chemother. 67, 1623–1626.

Fu, Y., Zhou, J., Zhou, H., Yang, Q., Wei, Z., Yu, Y., Li, L., 2010. Wide dissemination of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* clonal complex 22 in multiple cities of China. J. Antimicrob. Chemother. 65, 644–650.

Grosso, F., Carvalho, K.R., Quinteira, S., Ramos, A., Carvalho-Assef, A.P., Asensi, M.D., Peixe, L., 2011. OXA-23-producing *Acinetobacter baumannii*: a new hotspot of diversity in Rio de Janeiro? J. Antimicrob. Chemother. 66, 62–65.

Higgins, P.G., Lehmann, M., Seifert, H., 2010. 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 35, 305.

Higgins, P.G., Pérez-Llarena, F.J., Zander, E., Fernández, A., Bou, G., Seifert, H., 2013. OXA-235, a novel class D beta-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 57, 2121–2126.

Higgins, P.G., Poirel, L., Lehmann, M., Nordmann, P., Seifert, H., 2009. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 53, 5035–5038.

Irfan, S., Turton, J.F., Mehraj, J., Siddiqui, S.Z., Haider, S., Zafar, A., Memon, B., Afzal, O., Hasan, R., 2011. Molecular and epidemiological characterization of clinical isolates of carbapenem-resistant *Acinetobacter baumannii* from public and private sector intensive care units in Karachi, Pakistan. J. Hosp. Infect. 78, 143–148.

Jang, T.N., Lee, S.H., Huang, C.H., Lee, C.L., Chen, W.Y., 2009. Risk factors and impact of nosocomial *Acinetobacter baumannii* bloodstream infections in the adult intensive care unit: a case-control study. J. Hosp. Infect. 73, 143–150.

Jones, R.N., Ferraro, M.J., Reller, L.B., Schreckenberger, P.C., Swenson, J.M., Sader, H.S., 2007. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp.. J. Clin. Microbiol. 45, 227–230.

Karah, N., Sundsfjord, A., Towner, K., Samuelsen, Ø., 2012. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. Drug Resist. Update. 15, 237–247.

La Scola, B., Gundi, V.A., Khamis, A., Raoult, D., 2006. Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. J. Clin. Microbiol. 44, 827–832.

Lee, K., Yong, D., Jeong, S.H., Chong, Y., 2011. Multidrug-resistant *Acinetobacter* spp.: increasingly problematic nosocomial pathogens. Yonsei Med. J. 52, 879–891.

Lee, Y., Bae, I.K., Kim, J., Jeong, S.H., Lee, K., 2012. Dissemination of ceftazidime-resistant *Acinetobacter baumannii* clonal complex 92 in Korea. J. Appl. Microbiol. 112, 1207–1211.

Livermore, D.M., 2009. Has the era of untreatable infections arrived? J. Antimicrob. Chemother. 64, 29–36.

Martins, N., Martins, I.S., de Freitas, W.V., de Matos, J.A., Magalhães, A.C., Girão, V.B., Dias, R.C., de Souza, T.C., Pellegrino, F.L., Costa, L.D., Boasquevisque, C.H., Nouér, S.A., Riley, L.W., Santoro-Lopes, G., Moreira, B.M., 2012. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant *Acinetobacter baumannii*. Transpl. Infect. Dis. 14, 316–320.

Martins, N., Martins, I.S., de Freitas, W.V., de Matos, J.A., Girão, V.B., Coelho-Souza, T., Maralhães, A.C., Cacci, L.C., de Figueiredo, M.P., Dias, R.C., Costa-Lourenço, A.P., Ferreira, A.L., Dalla-Costa, L., Nouér, S.A., Santoro-Lopes, G., Riley, L.W., Moreira, B.M., 2013. Imported and Intensive Care Unit-Born *Acinetobacter baumannii* Clonal complexes: one-year prospective cohort study in intensive care patients. Microb. Drug Resist.. http://dx.doi.org/10.1089/mdr.2012.074.

Mostachio, A.K., Levin, A.S., Rizek, C., Rossi, F., Zerbini, J., Costa, S.F., 2012. High prevalence of OXA-143 and alteration of outer membrane proteins in carbapenem-resistant *Acinetobacter* spp. isolates in Brazil. Int. J. Antimicrob. Agents 39, 396–401.

Mugnier, P.D., Poirel, L., Naas, T., Nordmann, P., 2010. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. 16, 35–40.

Nigro, S.J., Hall, R.M., 2012. Tn6167, an antibiotic resistance island in an Australian carbapenem-resistant *Acinetobacter baumannii* GC2, ST92 isolate. J. Antimicrob. Chemother. 67, 1342–1346.

O'Shea, M.K., 2012. *Acinetobacter* in modern warfare. Int. J. Antimicrob. Agents 39, 363–375.

Peleg, A.Y., Seifert, H., Paterson, D.L., 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21, 538–582.

Pitcher, D.G., Sanders, N.A., Owen, R.J., 1989. Rapid extraction of bacterial genomic DNA with guadinium thiocyanate. Lett. Appl. Microbiol. 8, 151–156.

Pitout, J.D., Hossain, A., Hanson, N.D., 2004. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp.. J. Clin. Microbiol. 42, 5715–5721.

Ramírez, M.S., Vilacoba, E., Stietz, M.S., Merkier, A.K., Jeric, P., Limansky, A.S., Márquez, C., Bello, H., Catalano, M., Centrón, D., 2013. Spreading of AbaR-type genomic islands in multidrug resistance *Acinetobacter baumannii* strains belonging to different clonal complexes. Curr. Microbiol.. http://dx.doi.org/10.1007/s00284-013-0326-5.

Rasheed, J.K., Jay, C., Metchock, B., Berkowitz, F., Weigel, L., Crellin, J., Steward, C., Hill, B., Medeiros, A.A., Tenover, F.C., 1997. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. 41, 647–653.

Runnegar, N., Sidjabat, H., Goh, H.M., Nimmo, G.R., Schembri, M.A., Paterson, D.L., 2010. Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a single institution over a 10-year period. J. Clin. Microbiol. 48, 4051–4056.

Saladin, M., Cao, V.T., Lambert, T., Donay, J.L., Herrmann, J.L., Ould-Hocine, Z., Verdet, C., Delisle, F., Philippon, A., Arlet, G., 2002. Diversity of CTX-M beta-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. FEMS Microbiol. Lett. 209, 161–168.

Stietz, M.S., Ramírez, M.S., Vilacoba, E., Merkier, A.K., Limansky, A.S., Centrón, D., Catalano, M., 2013. *Acinetobacter baumannii* extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III. Infect. Genet. Evol. 14, 294-30.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33, 2233–2239.

Turton, J.F., Woodford, N., Glover, J., Yarde, S., Kaufmann, M.E., Pitt, T.L., 2006. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 44, 2974–2976.

Vaneechoutte, M., Dijkshoorn, L., Nemec, A., Kämpfer, P., Wauters, G., 2011. *Acinetobacter*, *Chryseobacterium*, *Moraxella*, and other nonfermentative gram-negative bacteria. In: Versalovic, J., Carroll, K., Funke, G., Jorgensen, J., Landry, M., Warnock, D. (Eds.), Manual of Clinical Microbiology. American Society for Microbiology Press, Washington DC, pp. 714–738.

Walther-Rasmussen, J., Høiby, N.J., 2006. OXA-type carbapenemases. Antimicrob. Chemother. 57, 373–383.

Woodford, N., Ellington, M.J., Coelho, J.M., Turton, J.F., Ward, M.E., Brown, S., Amyes, S.G., Livermore, D.M., 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp.. Int. J. Antimicrob. Agents 27, 351–356.

Zarrilli, R., Pournaras, S., Giannouli, M., Tsakris, A., 2013. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. Int. J. Antimicrob. Agents 41, 11–19.