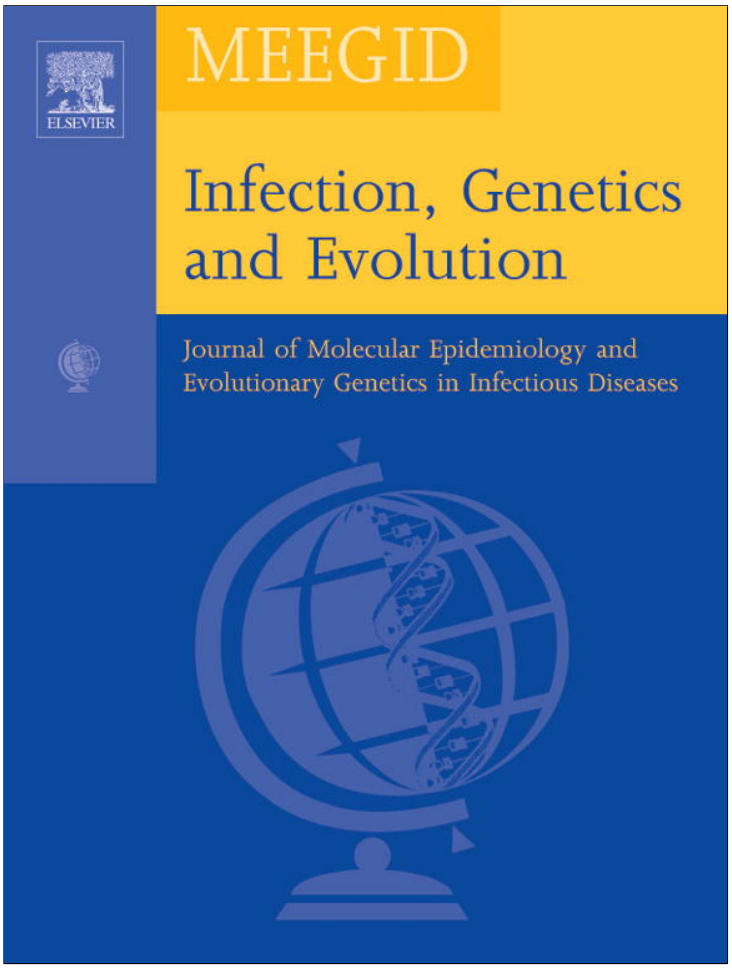

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Acinetobacter baumannii extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I–III

María Silvina Stietz [a], María Soledad Ramírez [a], Elisabet Vilacoba [a], Adriana Karina Merkier [a], Adriana Sara Limansky [b], Daniela Centrón [a], Mariana Catalano [a,*]

[a] Instituto de Microbiología y Parasitología Médica, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Tecnológicas (IMPAM, UBA-CONICET), Facultad de Medicina, Argentina
[b] Instituto de Biología Molecular y Celular de Rosario, Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Argentina

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ABSTRACT

As a way to contribute to the assessment of *Acinetobacter baumannii* clinical population structure, multilocus sequence typing (MLST) was performed in a collection of 93 isolates from Buenos Aires (1983–2012) and Rosario (2006–2009) hospitals. Sequence types (STs) were achieved by Bartual (B) and Institut Pasteur (P) schemes. PFGE typing, antimicrobial susceptibility assays, and the amplification of the OXA carbapenemase genes most prevalent in our region, were also performed.

e-Burst clustered the 25 STs^{B} (15 novels) into 5 clonal complexes (CC) and 5 singletons, and grouped the 18 STs^{P} (12 novels) into 3 CC and 4 singletons. Bartual scheme divided the $CC79^{P}$ into two groups. $CC113^{B}/CC79^{P}$ prevailed in Buenos Aires at least in 1992–2009, being responsible for epidemic and for endemic infections and acquiring the XDR (extensively drug-resistant) pattern throughout the years. While, $CC119^{B}/CC79^{P}$ was apparently present before the $CC113^{B}/CC79^{P}$domain. $CC103^{B}/CC15^{P}$ was the second most prevalent CC. Interestingly, $CC110^{B}/ST25^{P}$ apparently increased over the last years. Conversely, $CC109^{B}/CC1^{P}$ (international clone I) predominated in Rosario, although the presence of $CC113^{B}/CC79^{P}$, $CC103^{B}/CC15^{P}$ and $CC110^{B}/ST25^{P}$ was observed. Nineteen novel STs clustered in $CC79^{P}$, $CC15^{P}$, $CC113^{B}$, $CC109^{B}$ and $CC103^{B}$, suggesting their clonal expansion during persistence. PFGE typing proved transmission of strains intra- and inter-hospitals in each city. Except for one, all the recent isolates (2007–2012) harboured the $bla_{OXA\text{-}23\text{-}like}$. All isolates were susceptible to colistin. Tigecycline MIC^{90} was 1 mg/L and the rifampicin MIC > 512 mg/l was found among isolates in three hospitals.

In conclusion, the international clone II ($CC92^{B}/CC2^{P}$) was not found among our isolates. $CC113^{B}/CC79^{P}$, $CC103^{B}/CC15^{P}$, and $ST25^{P}$, suggested also as major components in the *A. baumannii* population together with the international clone I, were present in Buenos Aires and Rosario with different prevalence rate. Their recent isolates showed high distribution of the $bla_{OXA\text{-}23\text{-}like}$ as well as the XDR pattern.

1. Introduction

Acinetobacter baumannii has become one of the most complex causative agents of nosocomial infections due to its remarkable ability to survive on hospital surfaces and acquire antibiotic resistance (Maragakis and Perl, 2008, Peleg et al., 2008). Highly similar strains have been found at different geographical locations and at different time-points without a direct epidemiological link, leading to the assumption that a few genotypic groups are responsible for a large proportion of *A. baumannii* nosocomial infections (Dijkshoorn et al., 2007). Thus, isolates commonly referred to as the European clones EUI, EUII, and EUIII has predominated in many European countries since the 1990s (Dijkshoorn et al., 1996; van Dessel et al., 2004). Subsequently, EUI-III isolates were found worldwide, emphasising its epidemic potential, and re-named as "international clones I–III" (van den Broek et al., 2009; Diancourt et al., 2010; Karah et al., 2012). These clones were primarily delineated by AFLP and ribotyping (Dijkshoorn et al., 1996; Nemec et al., 2004; van Dessel et al., 2004). However, multilocus sequence typing (MLST), measuring the DNA sequence variations in seven housekeeping genes, proved to provide unambiguous typing data for global and long-term *A. baumannii* epidemiological studies (Bartual et al., 2005; Hamouda et al., 2010; Diancourt et al., 2010). Each isolate is characterised by its unique allelic profiles (se-

* Corresponding author. Address: Instituto de Microbiología y Parasitología Médica (IMPAM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Piso 12, C1121ABG Buenos Aires, Argentina. Tel.: +54 11 5950 9500x2181; fax: +54 11 4964 2554.
E-mail address: catalano@fmed.uba.ar (M. Catalano).

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quence type, ST), and by a stringent default setting STs with a single locus variant (SLV) are linked to the same clonal complex (CC) (Feil et al., 2004). Two MLST schemes are currently available for typing *A. baumannii* strains, one proposed by Bartual et al. (2005) and modified by Wisplinghoff et al. (2008); the other proposed by Institut Pasteur, France (Nemec et al., 2008; Diancourt et al., 2010). International clones I-III correspond to $CC92^{B}/CC2^{P}$, $CC109^{B}/CC1^{P}$and $CC187^{B}/CC3^{P}$, respectively; where B refers to Bartual and P to Institut Pasteur schemes (Karah et al., 2011). $CC109^{B}/CC1^{P}$ and $CC92^{B}/CC2^{P}$ were found to be spread in more than 30 countries, and $CC187^{B}/CC3^{P}$ was reported in six European countries, USA and Lebanon (Diancourt et al., 2010; Karah et al., 2012; Higgins et al., 2012). In several countries, a shift in the current *A. baumannii* population towards the international clone II, rather than the international clone I, has been reported (Nemec et al., 2008; Karah et al., 2011; Villalón et al., 2011). However, the broad spread of $CC113^{B}$, $CC103^{B}/CC15^{P}$, and $CC20^{B}$ suggests that they are also major components in the *A. baumannii* global population (Karah et al., 2012). Several CCs or STs are geographically restricted to one country and could include isolates favoured by the acquisition of specific resistance determinants (Giannouli et al., 2010; Ho et al., 2010; Karah et al., 2012). Thereby, *A. baumannii* outbreaks in four Mediterranean countries were caused by the spread of isolates belonging to $ST2^{P}$, and to a lesser extent, $ST83^{P}$ and $ST78^{P}$ were present in Turkey and Italy respectively, probably favoured by the acquisition of blaoxa-$_{58\text{-like}}$ genes (Giannouli et al., 2010; Di Popolo et al., 2011; Carretto et al., 2011).

In the *A. baumannii* local and short-term epidemiology investigations, PFGE (pulsed-field gel electrophoresis), MLVA (multiple-locus variable number tandem repeat analysis) and rep-PCR (repetitive sequence-based PCR) seem to be complementary to MLST (Hamouda et al., 2010; Villalón et al., 2011; Pourcel et al., 2011; Turton et al., 2011; Minandri et al., 2012; Mammina et al., 2012).

In order to contribute to the assessment of *A. baumannii* clinical population structure, this study was aimed to investigate the lineages of isolates circulating in Buenos Aires during 1983–2012 and in Rosario during 2006–2009. In addition, the distribution of the OXA carbapenemase-encoding genes prevalent in our region, and the inter-hospital transmission of strains were also evaluated.

2. Material and methods

2.1. Bacterial isolates

A total of 93 *A. baumannii* clinical isolates were included in this study. Seventy-six isolates were collected from seven hospitals in Buenos Ares city. Two isolates represented two outbreaks at hospitals H1 and H2, respectively (Quelle and Catalano, 2001) and forty-one isolates were from patients at H3, H4, H5 and H7, under an endemic setting. The other 33 isolates were from colonised and infected patients, personnel hand-carriage and environmental sources, selected over 159 isolates obtained during an endemic surveillance study performed at H6 in 2003 (Barbolla et al., 2008). Seventeen isolates were from infected patients at five different hospitals in Rosario (located at 300 km from Buenos Aires city, in the province of Santa Fe), obtained in 2006–2009 (Table 1).

2.2. Bacterial identification and PFGE

Genomic species identification was carried out by ARDRA (amplified ribosomal DNA restriction analysis) (Vaneechoutte et al., 1995). Macrorestriction was performed with ApaI (Promega Corporation, Madison, WI), and DNA digested fragments were separated in a CHEFDR III system (Bio-Rad, Richmond, CA) as described previously (Quelle and Catalano 2001). Similarities among PFGE patterns were calculated by the Dice coefficient method as described previously (Quelle and Catalano 2001). Clustering was performed by the unweighted-pair group method using average linkages (UPGMA); matrix was achieved by PyElph version 1.4 (Pavel and Vasile, 2012) and bootstrapping by DendroUPGMA. (http://genomes.urv.cat/UPGMA). A cut-off similarity value of 75% that correlated with $\geqslant 7$ bands difference was considered to delineate epidemiological unrelated strains (Tenover et al., 1995; Quelle and Catalano, 2001; Seifert et al., 2005).

2.3. Antimicrobial susceptibility

Minimum inhibitory concentration (MIC) to ampicillin/sulbactam, piperacillin/tazobactam, cefepime, ceftazidime, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, ofloxacin, minocycline and colistin was determined by agar dilution method following the Clinical and Laboratory Standards Institute recommendations (CLSI, 2011), and isolates were classified as non-MDR (multi-drug-resistant), MDR and XDR (extensively drug-resistant) according to Magiorakos et al. (2012). Regarding the epidemiological cut-off value, defined as the MIC value identifying the upper limit of the wild type population (http://www.eucast.org/), isolates with imipenem MIC of 1–8 mg/l and meropenem MIC of 2–8 mg/l were categorised as low level carbapenem resistant *A. baumannii* (LCRAB). Rifampicin and tigecycline MICs were also performed by agar dilution method and MIC^{50} and MIC^{90} were calculated.

2.4. MLST

MLST was performed according to the two schemes available by sequencing the internal region of *glt*A, *gyr*B, *gdh*B, *rec*A, *cpn*60, *gpi* and *rpo*D (http://pubmlst.org/abaumannii/); and *fus*A, *pyr*G, *rpo*B and *rpl*B in addition to *cpn*60, *glt*A and *rec*A genes (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). PCR products were purified with the NucleoSpin purification kit (Macherey–Nagel, Germany) according to the manufacturer's instructions. Nucleotide sequences of both chains were obtained using an ABI 373 DNA sequencer (Applied Biosystems, SA, Argentina) or by submitting purified PCR products to Macrogen (Macrogen Inc., Seoul, Korea). The new alleles and STs identified in this study were submitted to both Databases.

2.5. Data analysis

The eBurst program was used to survey the relationship between new and existing STs (Feil et al., 2004). For this purpose, several new STsB or alleles were designed with higher numbers than those present in Databases because they were recently submitted. The new STsP were codified by the Institut Pasteur's MLST *A. baumannii* working group. The analysis was performed under stringent (minimum of six shared alleles) grouping parameters. MEGA5 (Tamura et al., 2011) was used for neighbour-joining phylogenetic analysis using the concatenated sequences of the housekeeping genes proposed by each and by both MLST schemes. For the last analysis, the 11 housekeeping genes sequences were concatenated following the *A. baumannii* ATCC 17978 genome order. For this purpose, an arbitrary code was assigned to the combined allelic profiles (Table 1). Evolutionary distances were computed using the Jukes–Cantor method. Phylogeny was tested by the Bootstrap method with 1000 replications.

2.6. Molecular detection of bla_{OXA} carbapenemase genes

$bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$ and bla_{OXA143}, the most prevalent OXA carbapenemase genes in our region (Merkier et al., 2008; Gales

Table 1
Allelic Profiles and PFGE type found in the 93 *A. baumannii* Isolates.

CODE[a]	Allelic profile Bartual[b]	ST[B]	CC[Bd]	Allelic profile Pasteur[c]	ST[P]	CC [Pd]	PFGE	No of isolates (%)	
								Bs. As[e]	Rosario[e]
1	1-15-8-10-28-3-3-32	99	113	26-2-2-2-29-4-5	79	79	I	4 (5.2%)	-
2	1-15-8-10-28-55-32	100	113	26-2-2-2-29-4-5	79	79	I	8 (10.5%)	-
3	1-15-8-10-28-56-32	113	113	26-2-2-2-29-4-5	79	79	I[f]	25 (30.8%)	-
4	1-15-8-10-28-56-32	113	113	26-2-2-2-29-1-5	169	79	I	1 (1.3%)	-
5	1-15-8-10-28-56-32	113	113	26-4-2-2-29-4-5	170	79	XV	-	1(5.9%)
6	1-15-8-10-28-56-32	113	113	26-16-2-2-29-4-5	171	79	I	1 (1.3%)	-
7	1-15-8-10-28-57-32	114	113	26-2-2-2-29-4-5	79	79	I	5 (7.6%)	-
8	1-15-8-10-28-57-32	114	113	26-2-2-2-29-1-5	169	79	I	1(1.3%)	-
9	1-15-8-10-28-56-9	212	113	26-2-2-2-29-4-5	79	79	I	1 (1.3%)	-
10	1-17-8-10-28-56-32	603	113	26-1-2-2-29-4-5	175	79	I	-	1 (5.9%)
11	1-12-8-10-28-57-32	610	113	26-2-2-2-29-4-5	79	79	I	–	1 (5.9%)
12	1-15-12-6-28-59-40	119	119	26-1-2-2-9-1-5	166	79	III	1 (1.3%)	-
13	1-15-12-6-28-59-40	119	119	26-2-2-2-9-1-5	167	79	IV	1(1.3%)	-
14	1-15-12-6-28-59-40	119	119	26-4-2-2-9-1-5	109	79	III	3 (4.0%)	-
15	1-15-12-6-28-3-38	605	Singleton	26-4-2-2-9-1-5	109	79	III	1 (1.3%)	-
16	1-47-8-10-28-4-32	210	Singleton	26-16-2-2-29-4-5	171	79	I	1 (1.3%)	-
17	10-12-4-11-4-9-5	109	109	1-2-1-1-5-1-1	19	1	III, XIV[g], XV[g]	1 (1.3%)	3 (17.6%)
18	10-12-4-11-4-9-5	109	109	1-1-1-1-5-1-1	1	1	III, IV, XIV[g]	3 (4.0%)	2 (11.8%)
19	10-12-4-11-12-9-5	216	109	1-1-1-1-5-1-1	1	1	XIV, XV[g]	–	2 (11.8%)
20	10-17-8-11-4-9-5	604	109	1-1-1-1-5-1-1	1	1	XIII	1 (1.3%)	-
21	10-12-4-11-12-707-5	606	109	1-1-1-1-5-1-1	1	1	XVII	–	1 (5.9%)
22	10-12-4-11-4-56-5	611	109	1-1-1-1-5-1-1	1	1	XV	–	2 (11.8%)
23	10-12-4-11-12-57-5	612	109	1-1-1-1-5-1-1	1	1	XIV	–	1 (5.9%)
24	10-12-8-11-4-9-5	602	109	1-2-1-1-5-1-5	174	1	III	1 (1.3%)	-
25	10-12-4-11-28-9-5	601	109	26-4-1-1-5-1-1	173	1	XVa	–	1 (5.9%)
26	12-12-12-1-29-3-39	607	103	6-6-8-2-3-5-4	15	15	IV	1 (1.3%)	-
27	12-17-12-1-29-3-7	609	103	6-6-8-2-3-5-4	15	15	IV	1 (1.3%)	-
28	12-17-12-1-29-3-39	103	103	6-6-8-2-3-5-4	15	15	IV	4 (5.2%)	1 (5.9%)
29	12-17-12-1-29-3-39	103	103	6-6-8-1-3-5-4	165	15	IV	1 (1.3%)	-
30	1-15-2-28-1-52-32	110	110	3-3-2-4-7-2-4	25	Singleton	XVI	7 (9.2%)	1 (5.9%)
31	2-708-40-12-1-3-5	608	Singleton	3-3-6-2-4-1-29	177	Singleton	IV	2 (2.6%)	–
32	1-47-62-39-4-4-41	600	Singleton	1-16-2-6-18-4-4	172	Singleton	I	1 (1.3%)	–
33	1-52-62-39-4-4-38	211	Singleton	1-2-2-2-18-4-4	176	Singleton	I	1 (1.3%)	–

[a] Arbitrary number used to designate unique combination obtained by the two MLST schemes.
[b] Allelic profiles, ST (sequence type) and CC (clonal complex), using the *Acinetobacter baumannii* MLST Databases of University of Oxford, UK (http://pubmlst.org/abaumannii). Alleles order: *gltA-gyrB-gdhB-recA-cpn60-gpi-rpoD*. Novel ST210, ST211 numbers were codified by the University of Oxford, UK working group, those numbered 600–611 were submitted but were not codified, yet.
[c] Allelic profiles, ST and CC using the *Acinetobacter baumannii* MLST Databases of Institut Pasteur, France (http://www.pasteur.fr/mlst). Alleles order: *cpn60-fusA-gltA-pyrG-recA-rplB-rpoB*. Novel ST numbers were codified by the Institut Pasteur's MLST *A. baumannii* working group.
[d] CC determined by eBurst (http://eburst.mlst.net/).
[e] Bs. As: Buenos Aires city; Rosario: Rosario city, Santa Fé.
[f] One isolate showed PFGE type IV, and the other two isolates PFGE type XI (Table 2).
[g] PFGE of isolates recovered only from Rosario.

et al., 2012; Mostachio et al., 2012), were investigated by PCR amplification using specific primers as described previously (Merkier et al., 2008; Higgins et al., 2010). The reaction was also carried out in the LCRAB isolates.

3. Results

As shown in Table 1, 25 STs[B] (including 15 novel STs, and two novel alleles) and 18 STs[P] (with 12 novels) were identified. The e-Burst analysis clustered the STs[B] in five CCs and five singletons; and arrayed the STs[P] in three CCs and four singletons (Table 1, Fig. 1A). Through the combination of the allelic profiles of the two MLST schemes, 33 ST patterns were distinguished (Table 1). The neighbour-joining trees, using the concatenated allele sequences for each MLST scheme, revealed a similar eBurst picture (left and middle trees, Fig. 1B). The same analysis, using the concatenated sequences of the 11 MLST genes, clearly showed the split of the CC79[P] in two different groups, where isolates CC113[B] and ST119[B] were located respectively (right tree, Fig. 1B). In the same way, CC1[P] was split in two monophyletic branches by Bartual scheme (right tree, Fig. 1B). The removal of the *gyr*B and *gpi* sequences from the analysis amended to 26 the number of allelic patterns. However, the divergence between ST119[B]/CC79[P] and CC113[B]/CC79[P] was retained and minor changes in the CC113[B]/CC79[P] group were observed (right tree, Fig. 1B). On the contrary, in the CC109[B]/CC1[P] *gpi* sequences removal wiped out one monophyletic group (right tree, Fig. 1B) CC103[B]/CC15[P] was similarly pictured with and without *gyr*B and *gpi* sequences inclusion (right tree, Fig. 1B).

The CC113[B]/CC79[P] was found in 46/76 isolates from Buenos Aires. These isolates were related to outbreaks (H1 and H2), as well as to endemic or sporadic infections (H3, H4, H5 and H6) (codes 1–11) (Table 2). Isolates from outbreak at H2 and those recovered in 1995 from endemic infections at H3 were non-MDR (susceptible to ampicillin/sulbactam, piperacillin-tazobactam, cefepime, ceftazidime, imipenem, meropenem, ciprofloxacin, ofloxacin, minocycline, and colistin: MIC_{90}: 4/2 mg/l, 32 mg/l, 1 mg/l, 4 mg/l, 0.5 mg/l, <0.5 mg/l, 0.5 mg/l, 1 mg/l, 2 mg/l, 1 mg/l, respectively). Isolates recovered from the outbreak at H1 shared a similar susceptibility pattern except for resistance to piperacillin-tazobactam and ceftazidime (>256 mg/l and 32 mg/l, respectively); and isolates from endemic infections at H5 in 2002 added fluoroquinolone resistance (MIC: 32–64 mg/ml).In turn, isolates belonging to the same CCs recovered

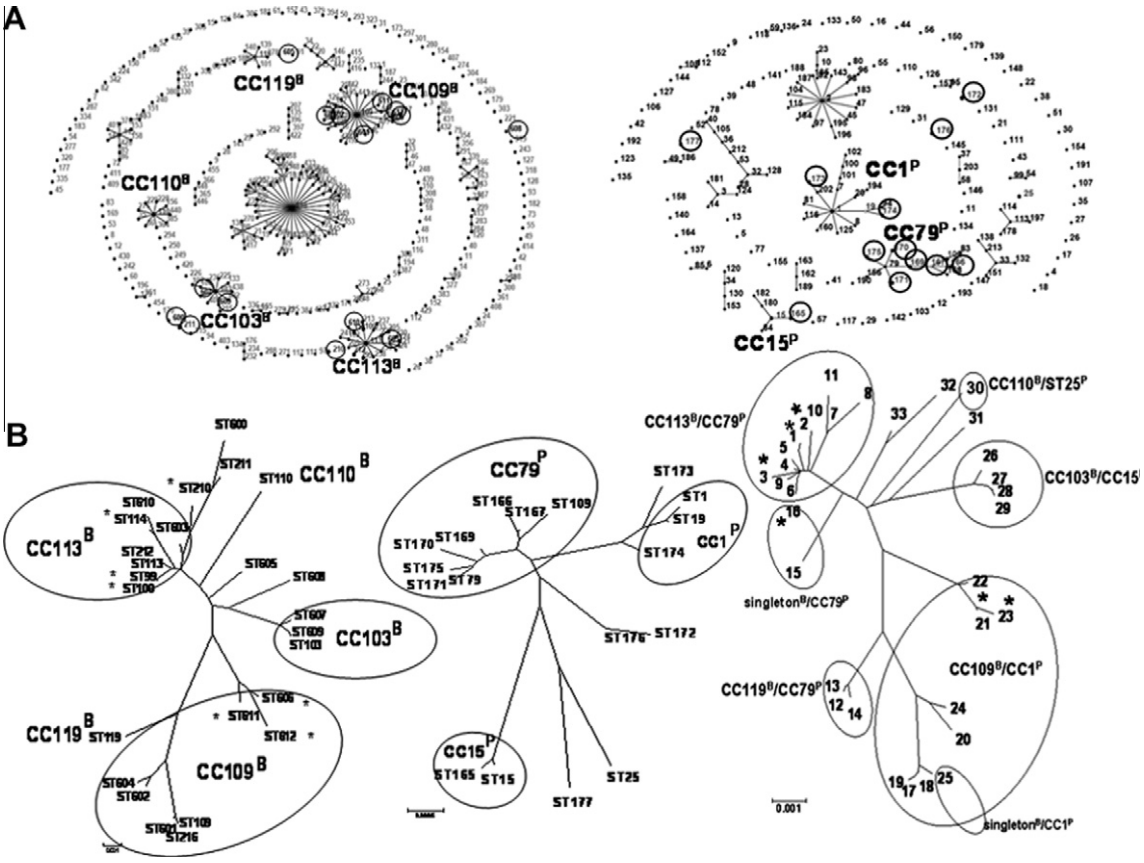

Fig. 1. Relatedness of *A. baumannii* sequence types (STs). (A) e-Burst analysis. The superscript B indicated the results obtained using University of Oxford, UK Database (http://pubmlst.org/abaumannii/); and superscript P those using Institut Pasteur database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). The novel STs were circled. (B) Neighbour-joining phylogenetic analysis. Left tree, using the University of Oxford, UK Database concatenated sequences. Middle tree, using the Institut Pasteur Database concatenated sequences. Right tree, using the concatenated sequences of the housekeeping genes of both schemes. Arbitrary numbers were assigned to the combinations obtained by the two MLST schemes. These numbers are depicted in Table 1. *, Allelic profiles which disappeared when *gyr*B and/or *gpi* sequences were removed from the analysis.

from H4 and H6 in 2002 and 2003, respectively, as well as isolates from H3 in 2006 were mainly XDR, with a MIC range to imipenem and meropenem of 8–32 mg/l (codes 1–4) (Table 2). In Rosario, CC113^{B}/CC79^{P} was identified only in 3/17 isolates, showing also a XDR pattern (Table 2). We ignore whether isolates of this lineage were present or absent in Rosario hospitals in 1980s and/or 1990s.

ST119^{B}/CC79^{P} were only identified in H3 (Buenos Aires) during 1983–1994 and all isolates were MDR (codes 12–14) (Table 2).

The international clone I (CC109^{B}/CC1^{P}) was identified in 11/17 isolates recovered in Rosario, all of them were XDR. In Buenos Aires, 6/76 isolates were clustered in this CC, showing MDR or XDR patterns according to the years of isolation (codes 17–24, Table 2) CC103^{B}/CC15^{P} was mainly identified in isolates from Buenos Aires also showing a diverse susceptibility pattern throughout the years of isolation (codes 25–29) (Table 2).

The first isolate ST110^{B}/ST25^{P} was recovered from H8 in Rosario in 2007. In Buenos Aires, the only three isolates analysed from H7 recovered in 2011 and the four isolates from H4 obtained in 2012 showed this alelic profile too. All isolates ST110^{B}/ST25^{P} were XDR (Table 2).

The 93 isolates were susceptible to colistin, and only three of them were resistant to minocycline. The MIC^{50} and MIC^{90} to tigecycline were 0.25 mg/l and 1 mg/l, respectively. Regarding rifampin, the MIC^{50} was 4 mg/l and the MIC^{90} > 512 mg/l. The MIC > 512 mg/l was shown mainly by isolates from H6 and H8 as well as by those recovered from H4 in 2010 and 2012.

The distribution of OXA carbapenemase genes exposed the prevalence of $bla_{\text{OXA-23-like}}$ among the isolates studied, being present in 56/90 (62.2%) isolates. The $bla_{\text{OXA-58-like}}$ gene was detected only in 11 isolates, recovered mainly from H6 (Table 2). Five isolates were positive for the amplification of both genes (Table 2). The presence of $bla_{\text{OXA-143}}$ was not detected. Of note, MDR strains that are negative for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143}}$ should be further characterised through the analysis of ISAba sequences, but this does not fall into the aims of the manuscript.

PFGE categorised the 93 isolates in 9 defined types (Tables 1 and 2, Fig.1S). Almost all the isolates with PFGE type I belonged to CC113^{B}/CC79^{P}, those from CC103^{B}/CC15^{P} exhibited PFGE type IV, and PFGE type XVI was shown only by CC110^{B}/ST25^{P} isolates. Conversely, CC109^{B}/CC1^{P} isolates showed diverse PFGE types (Tables 1 and 2). Therefore, this technique distinguished the isolates of the international clone I recovered from Buenos Aires and Rosario hospitals.

4. Discussion

The low levels of polymorphism found among clinical isolates worldwide may indicate that *A. baumannii* has recently experienced a severe bottleneck (a reduction in population size), elapsing a little time to accumulate diversity again (Diancourt et al., 2010). One hypothesis would be that the bottleneck was a consequence of a narrow ecological niche, as this species seems relatively rare in

Table 2
Multilocus sequence typing, PFGE, source, susceptibility pattern, OXA carbapenemase type, and year of isolation of *Acinetobacter baumannii* isolates.

Hospital[a]	ST CODE	PFGE	Isolate sources[b]	No. of isolates	Antibiotype[c]	$bla_{\text{OXA-23-like}}$	$bla_{\text{OXA-58-like}}$	Year of isolation
H1	1	I	Outbreak	1	MDR	–	–	1992
H2	1	I	Outbreak	11	Non-MDR	–	–	1995
H3	14	III	Endemic (I)[c]	3	MDR	–	–	1983, 93, 94
	13	IV	Endemic (I)	1	MDR	–	–	1987
	12	III	Endemic (I)	1	MDR	–	–	1992
	16	I	Endemic (I)	1	MDR	–	–	1992
	3	I	Endemic (I)	1	Non-MDR	+	–	1995
	18	III	Endemic (I)	1	MDR	–	–	1994
	17	IV	Endemic (I)	1	MDR	–	–	1994
	28	IV	Endemic (I)	1	MDR	–	–	1994
	29	IV	Endemic (I)	1	MDR	–	–	1997
	32	I	Endemic (I)	1	MDR	–	–	1997
	31	IV	Endemic(I)	2	MDR	–	–	1997
	15	III	Endemic (I)	1	MDR (LCRAB)	+	–	2005
	3	I	Endemic (I)	1	MDR (LCRAB)	+	–	2005
	33	I	Endemic (I)	1	MDR	–	–	2005
	7	I	Endemic (I)	1	XDR	+	–	2006
	1	I	Endemic (I)	1	XDR	–	+	2006
H4	28	IV	Endemic (I)	1	MDR (LCRAB)	ND	ND	2000
	3	I	Endemic (I)	3	XDR	+	–	2002
	4	Ic	Endemic (I)	1	XDR	+	–	2002
	2	Ic	Endemic (I)	1	XDR	+	–	2002
	3	I	Endemic (I)	2	XDR	+	–	2009
	3	XI	Endemic (I)	2	XDR	+	+	2010
	30	XVIc	Endemic (I)	3	XDR	+	–	2012
H5	2	I	Endemic (I)	1	MDR	–	–	2002
	8	I	Endemic (I)	1	MDR	–	–	2002
	3	I	Endemic (I)	4	MDR	–	–	2002
H6	2	Ie	Endemic (E)	2	MDR[d]	+	–	2003
	3	Ie	Endemic (E)	1	XDR	+	–	2003
	2	If	Endemic (E)	3	XDR	–	+	2003
	2	If	Endemic (E)	1	MDR	ND	ND	2003
	2	If	Endemic (E)	1	MDR	–	+	2003
	3	If	Endemic (E, HC, C, I)	12	XDR	+	–	2003
	3	If	Endemic (I)	1	XDR	–	–	2003
	9	If	Endemic (E)	1	XDR	+	–	2003
	6	If	Endemic (E)	1	XDR	+	+	2003
	3	Ig	Endemic (C)	1	XDR	+	–	2003
	24	IIIa	Endemic (I)	1	XDR	+	+	2003
	17	IIIa	Endemic (HC)	1	XDR	+	+	2003
	17	IIIa	Endemic (C)	1	XDR	–	+	2003
	28	IVb	Endemic (E, HC)	2	MDR[e]	–	–	2003
	26	IVb	Endemic (I)	1	XDR	+	–	2003
	27	IVb	Endemic(I)	1	MDR[e]	–	–	2003
	3	IVb	Endemic (I)	1	MDR[e]	+	–	2003
	20	XIIIa	Endemic (HC)	1	MDR	–	–	2003
H7	30	XVIa	Endemic(I)	2	XDR	+	–	2011
	30	XVIb	Endemic(I)	1	XDR	+	–	2011
H8	19	XIVc	Endemic (I)	1	XDR	+	–	2008
	17	XIVc	Endemic (I)	1	XDR	+	–	2009
	18	XV	Endemic (I)	1	XDR	+	–	2008
	22	XV	Endemic (I)	2	XDR	+	–	2008
	18	III	Endemic (I)	1	XDR	+	–	2009
	30	XVI	Endemic (I)	1	XDR	+	–	2009
	21	XVII	Endemic (I)	1	XDR	+	–	2009
H9	11	Im	Endemic (I)	1	XDR	+	–	2005
	23	XIV	Endemic (I)	1	XDR	+	–	2006
	28	IV	Endemic (I)	1	XDR	–	–	2007
	18	XIV	Endemic (I)	1	XDR	+	–	2007
	5	XV	Endemic (I)	1	XDR	+	–	2007
	25	XVa	Endemic (I)	1	XDR	ND	ND	2007
H10	19	XV	Sporadic (I)	1	XDR	+	–	2006
H11	18	XV	Sporadic (I)	1	XDR	+	–	2006
H12	10	I	Sporadic (I)	1	XDR	+	–	2007

[a] H1–H7: Buenos Aires hospitals, H8-H12: Rosario hospitals.
[b] Isolate source. I: infected patient, C: colonised patient, HC: personnel hand-carriage, E: environment. The thirty-three isolates from H6 were selected among 159 isolates obtained during an endemic surveillance study performed in 2003 (Barbolla et al., 2008) and PFGE types and subtypes, susceptibility patterns and origin of isolation were considered in this selection.
[c] MDR: isolates resistant to $\geqslant 1$ agent in $\geqslant 3$ antimicrobial categories, XDR: isolates resistant to $\geqslant 1$ agent in all but $\leqslant 2$ categories (Magiorakos et al., 2012); LCRAB: low levels of resistance to carbapenems, isolates with imipenem MIC 1–8 mg/l and meropenem MIC 2–8 mg/l. All isolates were susceptible to colistin, and to minocycline except for three isolates of H7.
[d] Isolates with MIC to imipenem 8 mg/l, meropenem 0.5 mg/l, and resistant to the other antimicrobials assayed, except for minocycline and colistin.
[e] Resistant to ampicillin/sulbactam, piperacillin-tazobactam, gentamicin, fluoroquinolones, ceftazidime, susceptible to amikacin, cefepime, carbapenems, minocycline and colistin.

humans and almost never found in soil (Diancourt et al., 2010). In addition, the human immune system and antibiotic treatments are the main stress conditions and crucial evolutionary bottlenecks for clinically relevant bacteria (Martinez and Baquero, 2002; Karah et al., 2012). Among others, these factors could explain the apparent considerable capability of the international clones, especially CC92^{B}/CC2^{P}, for their wide spread (Karah et al., 2012) However, recent evidence shows that beyond the three early recognised international clones, many others have a wide geographical distribution. Also, the spread of an increasing number of singleton STs (e.g., ST25^{P}, ST17^{B}, ST19^{B}, ST73^{B}, ST184^{B}, and ST49^{P}) could be observed, which might indicate rapid spread within a limited period of time, not sufficient for genetic differentiation and the creation of clonal complexes (Diancourt et al., 2010; Karah et al., 2012). In the isolates studied by our group, 33 ST profiles were defined by both Databases schemes. These STs clustered mainly in four phylogenetic groups. The removal of *gyr*B and *gpi* genes sequences, considering their location on recombination hotspot regions of the *A. baumannii* genome (Hamouda et al., 2010; Snitkin et al., 2011), decreased in seven the number of STs. However, Bartual et al. (2005) scheme divided the CC79^{P} into two groups: CC113^{B}/CC79^{P} and CC119^{B}/CC79^{P}, with different epidemiological behaviour in our region. ST79^{P}, as a singleton, was earlier found in a defined region of Spain in 2004 as a minority clonal lineage in one province, although the frequency of isolation increased in 2005, spreading to other provinces (Villalón et al., 2011). We found six novel STs that clustered into CC79^{P}, five of them in isolates from Buenos Aires suggesting the local clonal expansion during the long persistence in our hospitals. CC113^{B}/CC79^{P} was prevalent among isolates from Buenos Aires at least during 1992–2009, responsible for epidemic and endemic infections and acquiring the XDR pattern over the years. Isolates of this lineage and PFGE type I found in six hospitals indicated a high cross transmission. The environmental persistence of CC113^{B}/CC79^{P} PFGE type I was proven in H6. In this hospital, the isolates studied represent those recovered during a 10-week prospective cohort study including surveillance cultures of newly admitted patients in the intensive care unit until discharge and the weekly collection of environmental and staff hand-carriage samples (Barbolla et al., 2008). CC113^{B}/CC79^{P} mainly PFGE type if isolates were recovered from all kinds of samples (Barbolla et al., 2008). CC113^{B}/CC79^{P} was found also to prevail among the 96 OXA-23-producing isolates from different hospitals in Rio de Janeiro, Brazil (Grosso et al., 2010). This CC was also identified among CRAB (carbapenem resistant *A. baumannii*) in hospitals from the United States, but the international clone II predominated among them (Adams-Haduch et al., 2011).

ST119^{B}/CC79^{P} isolates seems to be endemic in H3 before the emergence of carbapenem resistance. In addition, three isolates from one hospital in Santiago de Chile recovered in 1997–1998 belonged to this lineage with the novel ST168^{P} (data not shown). We have no data about the clonal lineage frequently found in this or other hospitals from Chile, but ST119^{B}/CC79^{P} did not appear to be exclusively from Buenos Aires.

The international distributed CC103^{B}/CC15^{P} (Karah et al., 2012) followed in prevalence to CC113^{B}/CC79^{P}. This CC was present at least since 1994, and novel SLVs of ST103^{B} and ST15^{P} were also found.

CC110^{B}/ST25^{P} isolates emerged apparently in Buenos Aires over the last years. In our study, CC110^{B}/ST25^{P} was first identified in one isolate from Rosario, recovered in 2009. However, Oxford Database denoted one isolate recovered in 1997 in Argentina (http://pubmlst.org/abaumannii/). ST25^{P} was described as a European clone associated to a prevalent carbapenem-resitance (Diancourt et al., 2010; Karah et al., 2012), and CC110^{B} was also recovered from Korea and the USA (http://pubmlst.org/abaumannii/, Karah et al., 2012). To our knowledge, the lineage characterization CC110^{B}/ST25^{P} has not been described.

The international clone I was present into lesser extent among isolates from Buenos Aires. Rosario hospitals presented a different panorama with the prevalence of the international clone I, at least in 2006–2009, and in this city with CC109^{B}/CC1^{P} PFGE type XV isolates, intra-hospitals transmission was observed. Regardless of the prevalence of international clone I in each city, SLV of several STs from CC1^{P} and CC109^{B} were found among isolates from Buenos Aires and Rosario, indicating clonal diversification.

We cannot affirm that isolates belonging to international clones II and III were not present in Buenos Aires or Rosario hospitals, because this study was not done prospectively or in a consecutive period of time during 1983–2012. Moreover, Hamouda et al. (2010) included one isolate from Buenos Aires belonging to the international clone II among the collection of isolates carrying $bla_{\text{oxa-51-like}}$ genes. However, none of the isolates studied belong to the international clones II and III defined by MLST. Considering the extensively worldwide spread of clone II, that even displaced the clone I in several countries, our finding in Rosario hospitals could reflect the earlier composition of *A. baumannii* population found in other geographical regions. Nevertheless, the absence of CC92^{B}/CC2^{P} in the collection of isolates from both cities and the presence of the same CCs, although been different in prevalence, suggested a diverse local *A. baumannii* population structure. We hypothesised that whether a shift occurs it would tend to be in favour of CC110^{B}/ST25^{P}.

Regarding the OXA carbapenemase gene content, we observed the prevalence and increase of bla_{OXA23} throughout the period studied. The first isolate with carbapenemase among those included here, was from 1995. Except for one, all the recent isolates (2007–2012) harboured bla_{OXA23}, showing the importance of this gene as a source for carbapemen resistance in our clinical setting as observed in other countries worldwide (Zarrilli et al., 2012). In some countries, OXA-23-producing *A. baumannii* have recently tended to replace those OXA-58-producing (Zarrilli et al., 2012). Even though our isolates were not recovered in consecutive periods to confirm this observation, $bla_{\text{OXA-58-like}}$ was detected mainly in isolates from H6 in 2003 and several of them carried both OXA-carbapenemases. A correlation between ST and presence of $bla_{\text{OXA-23}}$ or bla_{OXA58} like was not found. The lack of $bla_{\text{OXA-143}}$ detection suggested that up to date this OXA-carbapenemases is missing in Argentina (Mostachio et al., 2012).

The MIC value >512 mg/ml to rifampicin was shown by isolates recovered from particular hospitals rather than from a single CC, indicating the distribution of this high level of resistance among the diverse lineages. Likewise, the XDR pattern was not exclusively from one particular CC.

5. Conclusion

Our results showed that isolates belonging to CC113^{B}/CC79^{P} and CC103^{B}/CC15^{P} prevailed among isolates from Buenos Aires at least in 1992–2009, with the apparent emergence of CC110^{B}/ST25^{P} during 2011–2012. In contrast, the international clone I predominated in hospitals from Rosario at least since 2006. Nineteen out of the 27 novel STs found clustered in CC79^{P}, CC15^{P}, CC113^{B}, CC109^{B}, CC103^{B}, demonstrating the local diversification of STs from this CCs during their persistence.

International clones II and III were not found among the collection of isolates.

More studies, including the MLST method in isolates from different regions of the same country and from different South America countries, are needed to evaluate the incidence of the designed international clones in this continent.

Authors' contributions

MSS performed MLST and PFGE typing. SR, AKM and DC provided isolate collection from H3 and H4. AKM performed the susceptibility patterns of H3. ASL collected isolates from H7-H11 and determined their susceptibility patterns. EV and SR carried out OXA- carbapenemasas amplification MC determined the susceptibility patterns of H1, H2, H4, H5 and H6. MC designed the research project and carried out the analysis of the results.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2012.12.020.

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