

## SHORT REPORT

# Preferential carriage of class 2 integrons in *Acinetobacter baumannii* CC113 and novel singletons

M. S. RAMÍREZ<sup>1,2\*</sup>, S. MONTAÑA<sup>1</sup>, M. CASSINI<sup>3</sup> AND D. CENTRÓN<sup>1</sup>

<sup>1</sup> Instituto de Microbiología y Parasitología Médica (IMPAM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Argentina

<sup>2</sup> Center for Applied Biotechnology Studies, Department of Biological Science, California State University Fullerton, Fullerton, CA, USA

<sup>3</sup> Grupo GEMA, Departamento de Ciencias Básicas, Universidad Nacional de Luján, Luján, Buenos Aires, Argentina & Laboratorio de Biología del Comportamiento, IBYME, Ciudad Autónoma de Buenos Aires, Argentina

Received 23 October 2014; Final revision 6 January 2015; Accepted 8 January 2015;  
first published online 20 February 2015

## SUMMARY

Our understanding of the distribution of integrons associated with multidrug resistance in *Acinetobacter baumannii* isolates around the world remains incomplete. The association between the class 1 and 2 integron *A. baumannii*-positive isolates ( $n = 60$ ), recovered since 1982 from 11 Argentinean hospitals, and the circulating lineages, was investigated. While class 2 integrons were highly significantly associated with clonal lineage CC113<sup>B</sup>/CC79<sup>P</sup> ( $P = 0.009$ ) and novel singletons ( $P = 0.001$ ), class 1 integrons were found not to be associated with CC109<sup>B</sup>/CC1<sup>P</sup> or other lineages. The study reveals a differential distribution of class 2 integrons in lineages, and suggests that the prevalence of *intI2* in Argentina is related to the emergence of novel singletons in recent years and to the abundance of CC113<sup>B</sup>/CC79<sup>P</sup>, which has been the local dominant lineage for several decades.

**Key words:** *Acinetobacter*, integron, lineages.

*Acinetobacter baumannii* has emerged as a major relevant global nosocomial pathogen in the last two decades, causing a variety of serious infections such as pneumonia and bacteraemia, often associated with high mortality [1]. In a CDC report published last year, *A. baumannii* was considered as a microorganism of serious concern, since about the 63% of the isolates are multidrug resistant with about 500 deaths each year attributed to its infections in the USA [2].

The accumulation of antibiotic resistance mechanisms occurs frequently in this species, underlining its genomic plasticity to evolve to extensive multidrug resistance in the nosocomial environment. Class 1 and 2 integrons have been frequently found in a variety of resistance determinants [3, 4]. However, a clear differential distribution of both these integron classes in clinical isolates across continents has been reported by different groups with class 1 integrons being frequently identified from Europe, USA and Australia, while class 2 integrons are more common in several countries in South America [3–5].

This prompted us to investigate if there was a particular dissemination of integrons in different *A. baumannii* lineages. We therefore analysed the association

\* Author for correspondence: M. S. Ramírez, PhD, Department of Biological Science, California State University Fullerton, 800 N State College Blvd, Fullerton, CA 92831, USA.  
(Email: ramirez.mariosoledad@gmail.com)

Table 1. Origin, clonal lineage and integron content of 60 isolates of *Acinetobacter baumannii* from 11 hospitals

Strain	Year	Hospital/city	CC/pan-PCR pattern*	<i>intI1</i>	<i>intI2</i>	Reference
Ab102	1983	H1 <sup>H</sup> /Buenos Aires	CC119	–	+	[9]
Ab109	1987	H1/Buenos Aires	CC119	–	+	[7]
Ab103	1993	H1/Buenos Aires	CC119	–	+	[9]
AbU1	2008	H1/Montevideo	CC119	–	+	[7]
Ab311	2002	H3/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
72	2002	H3/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[9]
Ab49	2002	H3/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
Ab42	2002	H3/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	+	+	[7]
Ab171	2005	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
Ab172	2005	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
Ab173	2005	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
Ab177	2006	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[9]
Ab181	2006	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[9]
Ab182	2006	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[9]
Ab181b	2006	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[7]
Ab177	2006	H1/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[9]
Ab394	2007	H1/Rosario	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[6, 7]
66	2012	H10/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	[8]
179	2012	H10/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	+	–	[8]
41 384	2013	H11/Buenos Aires	CC113 <sup>B</sup> /CC79 <sup>P</sup>	–	+	This work
66 285	2012	H9/Buenos Aires	CC110	+	–	This work
AbC3	2014	H10/Buenos Aires	CC110	–	+	This work
Ab110	1986	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab144	1987	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab155	1994	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab144	1997	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab115	1998	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab185	2006	H1/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[7]
Ab304	2006	H3/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	+	[6, 7]
Ab325	2007	H1/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[6, 7]
Ab326	2007	H1/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	+	[6, 7]
Ab376	2007	H5/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[6, 7]
Ab49011	2008	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	–	+	[6, 7]
Ab49631	2008	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	+	[6, 7]
Ab50309	2008	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	–	+	[6, 7]
Ab65513	2009	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	–	+	[6, 7]
Ab65986	2009	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	–	+	[6, 7]
Ab66284	2009	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[6, 7]
Ab66485	2009	H6/Rosario	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	[6, 7]
42	2011	H8/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	This work
14*2	2014	H8/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	This work
14*5	2014	H8/Buenos Aires	CC109 <sup>B</sup> /CC1 <sup>P</sup>	+	–	This work
Ab152	1997	H1/Buenos Aires	CC92 <sup>B</sup> /CC2 <sup>P</sup>	–	+	[7]
Ab125	1995	H1/Buenos Aires	Singleton (A)	–	+	[7]
Ab156	1997	H1/Buenos Aires	Singleton (ST600)	–	+	[7]
Ab138	1997	H1/Buenos Aires	Singleton (ST608)	–	+	[7]
Ab139	1997	H1/Buenos Aires	Singleton (B)	–	+	[9]
Ab145	1997	H1/Buenos Aires	Singleton (ST605)	–	+	[7]
Ab136	2001	H1/Buenos Aires	Singleton (ST614)	+	–	[7]
Ab174	2005	H1/Buenos Aires	Singleton (C)	–	+	[7]
Ab175	2005	H1/Buenos Aires	Singleton (C)	–	+	[7]
Ab176	2005	H1/Buenos Aires	Singleton (C)	–	+	[7]
Ab178	2006	H1/Buenos Aires	Singleton (ST605)	+	+	[7]
Ab179	2006	H1/Buenos Aires	Singleton (ST605)	+	+	[7]
Ab287	2006	H1/Rosario	Singleton (ST217)	+	+	[6, 7]
11 498	2009	H7/Buenos Aires	Singleton (p133)	–	+	This work

Table 1 (cont.)

Strain	Year	Hospital/city	CC/pan-PCR pattern*	<i>intI1</i>	<i>intI2</i>	Reference
11 635	2010	H7/Buenos Aires	Singleton (p133)	+	–	This work
423	2011	H8/Buenos Aires	Singleton (p133)	–	+	This work
376	2011	H8/Buenos Aires	Singleton (p133)	–	+	This work
204	2012	H2/Buenos Aires	Singleton (p133)	+	+	This work

CC, Clonal complex; PCR, polymerase chain reaction.

\* <sup>B</sup> Bartual scheme; <sup>P</sup> Institut Pasteur scheme.

between the circulating clones in South America and the presence of class 1 and 2 integrases in *A. baumannii* isolates recovered from 1982 to 2014 from 11 hospitals. Some isolates included in the present work, were from previously published studies (Table 1) [3, 6–8] but hitherto had not been investigated for association of strain lineages with the presence of class 1 and 2 integrons.

A total of 168 *A. baumannii* isolates was investigated by polymerase chain reaction (PCR) amplification of *intI1* and *intI2* genes using specific primers [3, 9]. Sixty isolates were positive either for class 1 ( $n = 17$ ) or class 2 integrases ( $n = 35$ ) or both ( $n = 8$ ). Confirmatory DNA sequencing was performed on 23 of the 43 *intI2* amplicons, and one *intI1* amplicon [3]. All *intI2* amplicons possessed the typical internal stop codon [3, 10], and the *intI1* amplicon harboured one of the most common alleles of *intI1* [9]. Isolates with class 1, or class 2 integrons were identified respectively in 9 and 10 of the 11 hospitals studied. Coexistence of both classes of integrons in the same strain was found in five hospitals from two cities, Buenos Aires and Rosario. Overall these results evidence the widespread presence of these genetic elements in nosocomial settings in Argentina.

The lineages of these 60 *intI*-positive isolates were determined by a pan-PCR assay [11] and multilocus sequence typing [12] which revealed the presence of six defined clonal complexes (CC) and at least nine singletons (Table 1) Statistical analysis was undertaken by the sign test which is a non-parametric test to confirm a zero median difference between continuous distributions of two random pairs. This showed high significant associations between *intI2* and the CC113 lineage ( $P = 0.003$ ) as well as between *intI2* and the novel singletons ( $P = 0.01$ ). In fact, 15/16 isolates of CC113<sup>B</sup>/CC79<sup>P</sup> and 15/17 isolates belonging to novel singletons harboured *intI2*.

Regarding class 1 integrons, 16 of the 25 *intI1*-positive isolates were assigned to lineage

CC109<sup>B</sup>/CC1<sup>P</sup>. Only two isolates of CC113 harboured a class 1 integron and the other six *intI1*-positive isolates corresponded to singletons, and one to CC110. No statistical association between CC109<sup>B</sup>/CC1<sup>P</sup> and class 1 integrons ( $P = 0.052345$ ) was found. However, CC109 was infrequently reported in Argentinean isolates recovered during the period 1982–2010 [12]. Interestingly, eight isolates possessed both integrons and belonged to different CCs or singletons; three were of lineage CC109<sup>B</sup>/CC1<sup>P</sup>, one of CC113<sup>B</sup>/CC79<sup>P</sup>, and five of different singletons (Table 1). These last results suggest that novel singletons may play a role in the double acquisition of integrons.

In conclusion, we describe a marked association between *intI2* and CC113<sup>B</sup>/CC79<sup>P</sup>, which is the most frequently isolated lineage of *A. baumannii* in Argentinean isolates during the years 1992–2009 and this explains the hitherto high frequency of *intI2* observed in isolates from Argentina [3, 6, 7, 12]. In addition, several novel singletons frequently containing *intI2*, some harbouring both classes of integrons, were evident.

## ACKNOWLEDGEMENTS

M.S.R., and D.C. are members of the career investigator programme of CONICET, Argentina. This study was supported by grants PIP 11420100100152 (CONICET) and PICT 0120 (ANPCyT) awarded to M.S.R., and UBACyT 2014–2017 (UBA) and PICT 0014 (ANPCyT) awarded to D.C.

## DECLARATION OF INTEREST

None.

## REFERENCES

1. Roca I, et al. The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace. *Frontiers in Microbiology* 2012; **3**: 148.

2. **Centers for Disease Control.** Antibiotic resistance threats in the United States. 2013 (<http://www.cdc.gov/drugresistance/threat-report-2013/>).
3. **Ramirez MS, et al.** Novel insights about class 2 integrans from experimental and genomic epidemiology. *Antimicrobial Agents and Chemotherapy* 2010; **54**: 699–706.
4. **Turton JF, et al.** Detection and typing of integrans in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. *Journal of Clinical Microbiology* 2005; **43**: 3074–3082.
5. **Fonseca EL, et al.** Class 2 integrans in multidrug-resistant *Acinetobacter baumannii* circulating in different Brazilian geographic regions. *International Journal of Antimicrobial Agents* 2011; **38**: 95–96.
6. **Ramirez MS, et al.** Increasing frequency of class 1 and 2 integrans in multidrug-resistant clones of *Acinetobacter baumannii* reveals the need for continuous molecular surveillance. *International Journal of Antimicrobial Agents* 2011; **37**: 175–177.
7. **Ramirez MS, et al.** Spreading of AbaR-type genomic islands in multidrug resistance *Acinetobacter baumannii* strains belonging to different clonal complexes. *Current Microbiology* 2013; **67**: 9–14.
8. **Vilacoba E, et al.** Outbreak of extensively drug-resistant *Acinetobacter baumannii* indigo-pigmented strains. *Journal of Clinical Microbiology* 2013; **51**: 3726–3730.
9. **Nardelli M, et al.** Class 1 integrans in environments with different degrees of urbanization. *PLoS ONE* 2012; **7**: e39223.
10. **Hansson K, et al.** *IntI2* integrin integrase in Tn7. *Journal of Bacteriology* 2002; **184**: 1712–1721.
11. **Yang JY, et al.** Pan-PCR, a computational method for designing bacterium-typing assays based on whole-genome sequence data. *Journal of Clinical Microbiology* 2013; **51**: 752–758.
12. **Stietz MS, et al.** *Acinetobacter baumannii* extensively drug resistant lineages in Buenos Aires hospitals differ from the international clones I-III. *Infection, Genetics and Evolution* 2013; **14**: 294–301.