



Published in final edited form as:

Curr Microbiol. 2012 March ; 64(3): 290–293. doi:10.1007/s00284-011-0068-1.

Class 2 Integrons Dissemination Among Multidrug Resistance (MDR) Clones of *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii has emerged as a serious problem in the hospital environment at a global scale. Previous results from our laboratory showed a high frequency of class 2 integrons in *A. baumannii* strains from Argentina regarding the low rate of this element in *A. baumannii* isolates from the rest of the world. To reveal the current epidemiology of class 2 integrons, a molecular surveillance analyzing 78 multidrug resistant (MDR) *A. baumannii* isolates from Argentina and Uruguay was performed, exposing the presence of class 2 integron in the 36.61% of the isolates. Class 2 integron characterization showed that the typical Tn7::In2-7 array was present in 26 out of 27 *intI2* positive isolates. All *intI2* positive isolates contained at least one of the Tn7 transposition genes. In addition, we identified that 18 *intI2* positive isolates possessed the Tn7::In2-7 within the *attTn7* site. The molecular typing evidenced that clones I and IV that do not belong to widespread European clones I and II were found among the *intI2* positive isolates. Our results exposed the widely dissemination of class 2 integron among MDR *A. baumannii* isolates from Argentina and

Uruguay, also showing the persistence of two novel clones in our region, which could explain in part the high frequency of class 2 integron found in our region.

Introduction

In the last years, nosocomial infections caused by *Acinetobacter baumannii* have emerged as a significant problem all over the world [6]. This species has shown that could rapidly evolve to the pandrug-resistance [6, 10]. The fact that *A. baumannii* could be resistant to all available antibiotic to treat it, placed *A. baumannii* isolates in the focus of health-care and nosocomial infection control programs [5, 6]. Class 1 and 2 integron elements could be found in *A. baumannii* clinical isolates [1–3, 8]. However, the distribution of class 2 integrons appears to be variable in different continents [8, 12], showing a high frequency of class 2 integrons in *A. baumannii* clinical isolates from Argentina, Chile, and Brazil [1, 2, 7, 8].

The purpose of this study was to find out the current epidemiology of class 2 integrons from Argentina and also Uruguay—from where there were no previous reports—by analyzing 78 multidrug resistant (MDR) *A. baumannii* isolates collected during the years 2008–2010.

Materials and Methods

Bacterial Strains

Seventy-eight MDR *A. baumannii* isolates recovered during the period 2008–2010 from Argentina ($n = 58$) and Uruguay ($n = 20$) were used (Table 1). The isolates were identified using the standard biochemical tests, microbiological test strip (API20NE-BioMerieux), and molecular methods (ARDRA).

DNA Techniques

Total DNAs and PCR amplifications were carried out as previously described [9]. To reveal the occurrence of class 2 integrons and characterize them, specific primers for the class 2 integrase, for the different gene cassettes and also specific primers for the transposition genes of Tn7 were used as previously described [9]. Class 2 integron location was investigated using a set of primers annealing in the *attTn7Ab* site and a region of the Tn7 transposon [7].

Molecular Typing

Macrorestriction analysis was done using genomic DNA digested with *ApaI* (Promega), according to the procedure previously described [4]. Clonal lineage groups of the *intI2* positive *A. baumannii* isolates was also established by the multiplex PCR described by Turton et al. [11].

DNA Sequencing

Several PCR products were sequenced after purifying the DNA by using the Wizard SV Gel and PCR clean-up System kit according to the manufacturer's directions (Promega, USA). Sequencing was performed on both DNA strands, using an ABIPrism 3100 BioAnalyzer

equipment. The nucleotide sequences were analyzed using the Blast V2.0 software (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Results and Discussion

The PCR reactions for the *intI2* gene gave positive results in 27 MDR *A. baumannii* isolates (36.61%). From the 27 positive isolates, ten were from Uruguay, which corresponds to the 50% of *intI2* positive isolates among the Uruguayan isolates (10/20). This result is in accordance with the previous documented epidemiology in the Argentinean isolates up to the year 2007 [8]. Concerning the *intI2* positive isolates from Buenos Aires city ($n = 17$), representing the 29% among the *A. baumannii* isolates studied (17/58), we noticed a decrease in the percentage of isolates carrying class 2 integrons comparing with the previous result found in our *A. baumannii* isolates (51%). However, the dispersion of class 2 integrons remains higher than the frequency of class 1 integrons we have found in *A. baumannii* (2/78) [8]. These results are in agreement with the recently published study from Brazil, in which they found only a positive isolate for class 1 integron, being the 36% of the isolates positive for class 2 integrons [1]. In addition, our results exposed that the 67% of the class 2 integrons were inserted in the *attTn7Ab* site as reported before for other class 2 integrons [9].

The clonal lineage groups of the *intI2* positive *A. baumannii* isolates were investigated with the multiplex PCR described by Turton and co-workers [11]. All the isolates were negative for this multiplex PCR suggesting that the *intI2* positive circulating clones in Argentina and Uruguay did not correspond to the European clones (Table 1). The clonal relationship by PFGE analysis showed the presence of 3 clones I ($n = 22$), IV ($n = 4$), and III ($n = 1$) among the 27 *intI2* positive isolates. We have previously documented the wide dissemination of the prevalent clone I in Buenos Aires Hospitals since many years ago [4]. As it is shown in this study, the high prevalence of class 2 integrons among MDR *A. baumannii* isolates from Argentina and Uruguay could be justified since that 22 out of 27 *intI2* positive isolates belonged to the same clone (clone I).

In the characterization of the class 2 integron variable region, the typical In2-7 array (*dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA*) was found in 26 out of 27 (96%) isolates. In two isolates, AU16 and A10, the presence of two class 2 integrons was found (Table 1), enclosing the arrays *sat2-aadA1-orfX-ybfA-ybfB-ybgA*, *intI2-dfrA1*, and In2-7, *sat2-aadA1-orfX-ybfA-ybfB-ybgA*, respectively (Table 1). Both isolates belonged to the most dispersed *intI2* positive clone, the clone I, denoting the possibility of gene cassettes exchange content among class 2 integron structures. The In2-8, which we found widely disseminated among *intI2* positive *A. baumannii* isolates from Chile [7], was not found in the present survey.

The occurrence of the Tn7 transposition genes was also investigated, showing that at least one of the Tn7 transposition genes was present in all isolates, and only 48% of the *intI2* positive isolates contained the five genes of the transposition module. These results are in agreement with our previous obtained results, where we detected all Tn7 transposition genes in 51% (44/86) of the isolates [8].

The exposed results showed that the persistence and prevalence of the clone *I intI2* positive, not belonging to the European clones I and II, could explain the greater dispersion of class 2 integrons in isolates of *A. baumannii* circulating in Argentina and Uruguay regarding the low observed rate of these elements in the population of *A. baumannii* in the rest of the world.

Acknowledgments

M.S.R and D.C. are members of the Carrera del Investigador Científico, C.O.N.I.C.E.T., Argentina. E.V. has type I Fellowship of C.O.N.I.C.E.T. A.M. was supported by grant MHIRT 2T37MD001368 from the National Center on Minority Health and Health Disparities, National Institutes of Health. This study was supported by grant PICT 0690 and PICT 0354 from the ANPCyT to D.C. and M.S.R., respectively, Buenos Aires, Argentina.

Appendix

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Table 1
Description of the class 2 integron variable region (VR), *tns* gene composition, and chromosome localization in the 27 *intI2* positive isolates

Isolates	Country	Transposon and class 2 integron	VR	<i>tns</i> genes	Clone/lineage group ^a
A1544	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A1615	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A1680	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A4St	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A4	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A21	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
AU3A	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
AU2	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	IV/negative
AU3B	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
AU5	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	IV/negative
AU6	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	IV/negative
A39513	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A39265	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A95	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC, tnsB, tnsA</i>	I/negative
A1357	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC, tnsB, tnsA</i>	I/negative
A98	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC, tnsB, tnsA</i>	I/negative
A108C	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsB, tnsA</i>	I/negative
A1562	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsB, tnsA</i>	I/negative
A1570	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsB, tnsA</i>	I/negative
AU16	Uruguay	In2-3	<i>dfraI-sat2</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A10	Argentina	Tn7::In2-6	<i>sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>		
A1616	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsB, tnsA</i>	I/negative
A97	Argentina	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsA</i>	I/negative
AU11	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsD, tnsC, tnsA</i>	I/negative
AU15	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC, tnsB</i>	I/negative
AU18	Uruguay	Tn7::In2-7	<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC</i>	I/negative
			<i>dfraI-sat2-caadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsC</i>	III/negative

Isolates	Country	Transposon and class 2 integron	VR	<i>tns</i> genes	Clone/lineage group ^a
AU1	Uruguay	Tr17::ln2-7	<i>dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>	<i>tnsE, tnsB, tnsA</i>	IV/negative

A. A. *baumannii* Argentinian isolates, AU A. *baumannii* Uruguayan isolates, VR variable region of class 2 integron

^aMultiplex PCR to determine clonal lineage groups 1, 2, and 3 [10]