

Spreading of AbaR-type Genomic Islands in Multidrug Resistance *Acinetobacter baumannii* Strains Belonging to Different Clonal Complexes

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Spreading of AbaR-type Genomic Islands in Multidrug Resistance *Acinetobacter baumannii* Strains Belonging to Different Clonal Complexes

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Abstract In order to determine the occurrence of AbaR-type genomic island in multidrug resistant *Acinetobacter baumannii* (MDRAb) strains circulating in Argentina, Uruguay, and Chile, we studied 51 MDRAb isolates recovered from several hospitals over 30 years. AbaR-type genomic resistance islands were found in 36 MDRAb isolates since 1986 till now. MLST technique allowed us to identify the presence of four different Clonal Complexes (109, 104, 119, 113) among the positive AbaR-type island positive strains. This is the first description of AbaR-type islands in the CC104 and CC113 that are the most widespread Clonal Complexes in Argentina. In addition, PCR

mapping exposed different arrays to those previously described, evidencing the plasticity of this island. Our results evidence a widespread distribution of the AbaR-type genomic islands along the time in the MDRAb population, including the epidemic global clone 1 (GC1) as well as different clonal complexes to those already described in the literature.

Introduction

Over the past years, multidrug resistant *Acinetobacter baumannii* (MDRAb) isolates have been reported with increasing frequency, being mainly related to nosocomial infections [9, 18]. The growing incidence of multidrug resistant *A. baumannii* infections could be explained by its resistant phenotype, and also by its ability to survive in different environments, as care facilities and medical devices, for a prolonged length of time [10, 16]. The acquisition of resistance has been correlated with the large number of resistant determinants in the *A. baumannii* genome [1, 8]. In recent years, multidrug resistance structures named as AbaR have been reported invading the *comM* gene in *A. baumannii* [1, 5, 8, 12–14, 17, 19, 23, 26]. The AbaRs have been described in strains belonging to the epidemic global clones (GC) 1 and 2, which are known to have a globally widespread behavior [6, 14, 17, 23]. The common AbaR backbone involves five genes that constitute the so called transposition module (*orf1*, *tmiA*, *tmiB*, *orf2*, *orf3*), in addition to the *uspA* and *sul* genes [5, 19]. Recent publications highlighted the differences found among the AbaRs that are described in the GC1 and GC2 [5, 14, 17, 19, 23, 26]. It was shown that in the AbaR3-like islands, described in the GC1, the gene *uspA* was interrupted by the Tn6018 transposon, while in the AbaR4-like

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islands, this gene is intact. In addition, the AbaR4 derivatives have been described in the GC2 at the *comM* site and also in other genomic locations in the GC1 [1].

The aim of the present study was to determine the spreading and variability of AbaR genomic island in 51 non-epidemiologic related multidrug resistant *A. baumannii* (MDRab) strains.

Materials and Methods

Bacterial Strains

Fifty-one MDRab strains, selected from a collection of 250 MDRab strains, were chosen to evidence the occurrence of AbaR. Most isolates corresponded to Argentinean Hospitals ($n = 46$). We also added few samples of the neighboring countries, Uruguay ($n = 3$) and Chile ($n = 2$). The criteria to select the MDRab were that the strains have been recovered from different hospitals of Argentina ($n = 6$), Uruguay ($n = 1$), and Chile ($n = 1$), at different years (1982 through 2010) and belonged to different PFGE clones (I, II, III, IV, VI, VII, VIII, IX, XII, XIV) [3, 15, 20, 22]. The included strains exhibited the typical multidrug resistance profiles (CIP/AMK/GEN/CAZ/FEP/IPM/MEM, CIP/GEN/CAZ/FEP/IPM/MEM, CIP/AMK/GEN/CAZ) described in the *A. baumannii* strains [15, 21, 22].

DNA Techniques

Total DNA was extracted using the Master Pure DNA purification kit following manufacturer's instructions (Epicentre, Madison, WI, USA). Previously described specific primers (4F, 4R, 2F, 2R) [24] were used to evidence the occurrence of AbaR islands in the selected strains. AbaR-based PCR mapping reactions were used to determine the existence of partial related structures to the AbaR islands described in the literature [19, 24]. In each strain, 40 PCR reactions were performed using different combinations of primers (Table 2). PCR amplifications were carried out in 50 μ l volumes containing 10 ng of DNA, 10 μ l of 5 \times PCR buffer, 0.5 μ l of 10 \times deoxynucleoside triphosphate mix (2 mM each dATP, dCTP, dGTP, and dTTP), 2 μ l of each primer stock solution (2.5 pmol of each primer per μ l), and sterile distilled water. Taq DNA polymerase, according to manufacturer's instructions (Promega, Madison, USA), was added. The thermocycler used was from Perkin-Elmer Cetus, Emeryville, CA, and a three-step profile was utilized. The nucleotide sequence and coordinates of the primers used in the PCR mapping are listed in Table 2. As we do not have GC2 in our selected strains, we used the AbaR3 genomic island as reference for PCR mapping studies (Fig. 1a).

Sequence Analysis

Sequencing was performed on both DNA strands using an ABIPrism 3100 BioAnalyzer and Taq FS Terminator Chemistry (Taq FS, Perkin-Elmer). Sequences were examined and assembled with Sequencher 4.7 software (Gene Codes Corp.) and BLAST (version 2.0) software (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Molecular Typing

To define the sequence type (ST) and clonal complex (CC) of the AbaR-type positive isolates, MLST was carried out. Briefly, amplification and sequencing of the internal fragments of the seven housekeeping genes *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD* were performed according to Bartual et al. [4], in order to compare the obtained results with previously region data [11]. From the comparison of the sequence of each allele with existing sequences in the *A. baumannii* MLST database (<http://pubmlst.org/abaumannii/>), the STs were assigned according to the obtained profiles. The novels STs were submitted for their inclusion in the Oxford Database. The eBURST algorithm (<http://eburst.mlst.net/>) was used to assess the genetic relationship of STs with the most stringent definition of the groups by sharing alleles at six of seven loci [7]. For this purpose, novels STs were designed with higher numbers than those present in the Oxford, UK Databases.

Results and Discussion

There is no documentation of the distribution of the AbaR-type genomic island among *A. baumannii* strains from South America. To determine the spreading of AbaR-type genomic island in our population, we searched its presence in 51 MDRab selected strains from Argentina ($n = 46$), Uruguay ($n = 3$), and Chile ($n = 2$), using previously described primers for PCR reactions [19, 24], followed by sequencing confirmation (Table 2). In 36/51 MDRab strains (71 %) (34 from Argentina and 2 from Uruguay), the *comM* gene was disrupted by AbaR-type genomic islands. Almost all the isolates ($n = 34$) were positive for the 3' and the 5' PCR junction, with the exception of two isolates (Ab311 and Ab49) where the AbaR-type 3'-end was absent (Table 1).

MLST technique allowed us to identify 18 STs in the studied population, evidencing eight new STs and three new alleles (Table 1). The eBURST algorithm grouped our AbaR-type resistance islands positive strains in CC109, CC104, CC119, CC113 and seven singletons (Table 1). This result exposed the high dispersion of AbaR positive isolates in the GC1 (CC109) all around the world.

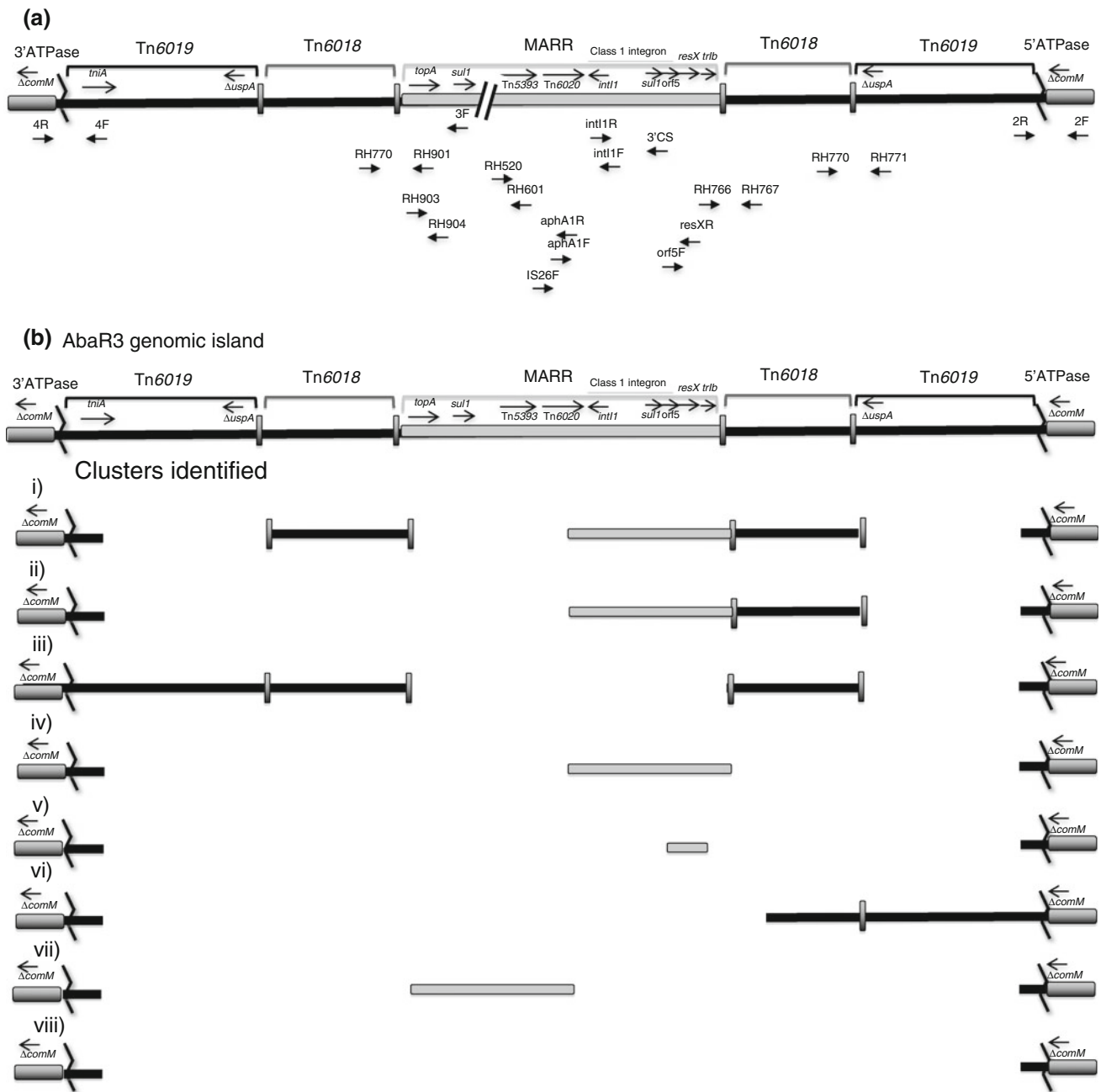


Fig. 1 a Schematic representation of the typical Aba3 genomic island structure from Adams et al. [1] used as reference for PCR mapping. Lines of different thicknesses and colors represent the different regions and elements. Vertical bars indicate the inverted repeats. Genes are shown by horizontal arrows (→) with the gene

name below. Location of the primers used in the study for PCR reactions are represented by small arrows (→). The figure is not to scale. b Representation of the eight clusters identified among the Aba3-like islands present in the 21 chosen *A. baumannii* AbaR-type positive strains

The main novelty of our results is the presence of AbaR-type genomic islands in novel CC (CC104, CC113, CC119).

Twenty-one AbaR-type positive strains including different CC were chosen and subject to further studies. As GC2 was not present in our isolates, the Aba3 genetic organization was used for the partial characterization of the AbaRs [1, 14]. In each strain, that contained AbaR island, 40 PCR

mapping reactions were performed using different combinations of primers as previously described (Table 2; Fig. 1a) [19, 24]. Among the Aba3-like islands, we identified eight clusters: (i) those that were positive for the reactions done to link the Tn6018-L and Tn6018-R with the MAAR region, also containing the class 1 integron and *sul1-orf5-resX-triB* genes (Ab304, Ab49631, Ab110, Ab144), (ii) those positive

Table 1 Description of the positive AbaR-type *A. baumannii* strains

Strains	Country	Hospital/city	Years	Clone	ST	CC	5'-ATPase junction ^a	3'-ATPase junction ^a
Ab163	ARG	H1/Buenos Aires	1982	IV	103	104	+	+
Ab130	ARG	H1/Buenos Aires	1984	IV	103	104	+	+
Ab110	ARG	H1/Buenos Aires	1986	VIII	109	109	+	+
Ab109	ARG	H1/Buenos Aires	1987	IV	119	119	+	+
Ab144	ARG	H1/Buenos Aires	1987	IV	NA	109	+	+
Ab104	ARG	H1/Buenos Aires	1992	I	210	Single	+	+
Ab155	ARG	H1/Buenos Aires	1994	IV	109	109	+	+
Ab133	ARG	H1/Buenos Aires	1996	IV	103	104	+	+
Ab149	ARG	H1/Buenos Aires	1996	IV	104	104	+	+
Ab156	ARG	H1/Buenos Aires	1997	I	NA	Single	+	+
Ab138	ARG	H1/Buenos Aires	1997	IV	NA	Single	+	+
Ab115	ARG	H1/Buenos Aires	1998	IV	109	109	+	+
Ab1454	ARG	H2/Buenos Aires	2000	IV	NA	Single	+	+
Ab136	ARG	H1/Buenos Aires	2001	IV	NA	Single	+	+
Ab311	ARG	H3/Buenos Aires	2002	I	100	113	+	-
Ab49	ARG	H3/Buenos Aires	2002	I	100	113	+	-
Ab42	ARG	H3/Buenos Aires	2002	I	100	113	+	+
Ab171	ARG	H1/Buenos Aires	2005	I	113	113	+	+
Ab172	ARG	H1/Buenos Aires	2005	IV	113	113	+	+
Ab185	ARG	H1/Buenos Aires	2006	III	109	109	+	+
Ab179	ARG	H1/Buenos Aires	2006	XII	NA	Single	+	+
Ab287	ARG	H1/Rosario	2006	XIV	217	Single	+	+
Ab304	ARG	H3/Rosario	2006	III	216	109	+	+
Ab325	ARG	H1/Rosario	2007	XIV	109	109	+	+
Ab326	ARG	H1/Rosario	2007	XIV	109	109	+	+
Ab376	ARG	H5/Rosario	2007	III	109	109	+	+
Ab394	ARG	H1/Rosario	2007	I	113	113	+	+
Ab49011	ARG	H6/Rosario	2008	XIV	NA	109	+	+
Ab49631	ARG	H6/Rosario	2008	III	216	109	+	+
Ab50309	ARG	H6/Rosario	2008	XIV	109	109	+	+
AbU1	URU	H1/Montevidéo	2008	IV	101	119	+	+
AbU2	URU	H1/Montevidéo	2008	IV	101	119	+	+
Ab65513	ARG	H6/Rosario	2009	III	NA	109	+	+
Ab65986	ARG	H6/Rosario	2009	III	109	109	+	+
Ab66284	ARG	H6/Rosario	2009	III	109	109	+	+
Ab66485	ARG	H6/Rosario	2009	III	109	109	+	+

ARG Argentina, URU Uruguay, NA ST not assigned yet

^a 5'-ATPase junction and 3'-ATPase junction by PCR reaction with specific primers 2F-2R and 4F-4R [24]

for Tn6018-R-MAAR region harboring the class 1 integron and *sul1-orf5-resX-trlB* genes (Ab287, Ab49631), (iii) those positive for *tniA*, *tniB*, Tn6018-L-MAAR, and Tn6018-R-MAAR junction region (Ab136), (iv) those positive for *orf5-resX* reaction also harboring class 1 integrons (Ab325, Ab376, Ab66285), (v) those only positive for *resX* gene (Ab326, AbU2, Ab109), (vi) those only positive for the junction region between Tn6018-R and Tn6019 (Ab49011,

Ab65513, Ab65986), (vii) those only positive for *top* gene and Tn6020 amplification (Ab155), and cluster (viii) corresponded to strains that gave negative results for all PCR reactions used to partially characterize the AbaR structure (Ab185, Ab179, Ab115, Ab42) (Fig. 1b).

Our results exposed a great heterogeneity of structures all of them being also different to the known AbaR-type islands previously reported in the literature [1, 8, 13, 19].

Table 2 Nucleotide sequences of the primers used for PCR mapping

Oligonucleotide	Sequence (5'–3')	Primer location	Coordinates (AN CP001182)	References
4R	AATCGATGCGGTGAGTAAC	<i>comM</i>	263266–263285	[24]
4F	TATCAGCAGAAAACGATGG	Tn6019	264111–264092	[24]
3R	CTGCTACGGCTGAAACATCC	<i>sulI</i>	323025–323044	[24]
3F	TGTACCTGCTGTCGTCTTCG	Tn6019	265769–265750	[24]
2R	TTGGGGATTCTGTCCGTAAG	Tn6019	326099–326118	[24]
2F	TCCATTTTACCGCCACTTTC	<i>comM</i>	326704–326685	[24]
RH770	CGATGCCCTAGAGAGAGTGCGC	Tn6018	278625–278646/321899–321920	[19]
RH771	TGTAATACTGGTGGTCGTAC	Tn6019	323512–323492	[19]
RH901	GCGGCTCTATCCCTAGTTCC	<i>top</i> (MARR)	280151–280132	[19]
RH766	TCCTGCGTCAAATCTGCTGTG	<i>trbI</i> (MARR)	319036–319057	[19]
RH767	CCTCCGATGTTTGGATATG	Tn6018	320469–320450	[19]
RH903	GGGCAAGGTGAAGAAGATCA	<i>topA</i> (MARR)	280045–280064	[19]
RH904	GTCTGATAGCTGGCGTCACA	<i>topA</i> (MARR)	281979–281960	[19]
aphA1R	CAAACCGTTATTCATTCGTGA	<i>aphA1b</i> (MARR)	309712–309732	[19]
aphA1F	AAACGTCTTGCTCGAGGC	<i>aphA1b</i> (MARR)	310172–310155	[19]
IS26F	ACCTTGATGGTGGCGTAAG	IS26 (MARR)	310923–310904	[19]
orf5F	ATATCGACGAGTTGTGC	Orf5 (MARR)	316618–316635	[2]
intI1F	CGAGGCATAGACTGTAC	<i>intI1</i> (MARR)	312669–312653	[2]
intI1R	TTCGAATGTCGTAACCGC	<i>intI1</i> (MARR)	311746–311763	[2]
sulF	GACGGTGTTCGGCATTCT	<i>sulI</i> (MARR)	315610–315627	[2]
aac(6')-IbF	GAAGAAGCACGCCCGAC	<i>aac(6')-Ib</i>	NC	This study
aadAr	CGCAGATCAGTTGGAAG	<i>aadA1</i> (MARR)	315034–315050	[2]
resXR	GTCCTGCTCCGGCGTGGACACGC	<i>resX</i> (MARR)	317840–317818	This study
RH601	GATGGAGCTGCACATGAACC	IS26 (MARR)	308059–308078	[19]
RH520	CATGCCCCAGCGGATACTTCAG	Tn5393 (MARR)	307883–307905	[19]

AN accession number, MARR multiple antibiotic resistance region, NC not correspond

As previously described, we observed different arrays and deletions compared to AbaR-type islands reported, supporting the high variability and plasticity of this element (Fig. 1b).

In addition, in seven strains belonging to the CC109 (GC1), we observed the presence of the typical class 1 integron *aacC1-orfP-orfQ-aadA1* inserted in the AbaR structure. In contrast, in the strains belonging to the CC104 and CC113, the most distributed clonal complexes of our countries [25], negative results were obtained for class 1 integrons, suggesting the absence of this element associated to AbaR islands.

The obtained data are, in part, in accordance with those published findings where usually strains belonging to GC1 were reported as harboring an AbaR-type resistance island containing integrons [19], although novel genetic arrays have been identified in our isolates. In addition, different arrays and deletions were found in the AbaR-type islands harbored by novel clonal complexes, supporting the high variability, plasticity, and the ability to disperse of this element. The AbaR-type genomic islands among the

A. baumannii population have been adapted among different lineages, along the time and around the world.

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