

Tn7::In2-8 dispersion in multidrug resistant isolates of *Acinetobacter baumannii* from Chile

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ABSTRACT

Acinetobacter baumannii is considered an important pathogen in our hospital environment having a well-known capacity to acquire different mechanisms of antibiotic resistance. Previous studies in our laboratory had exposed the high dispersion of class 2 integrons in this species. In the present study, we analyzed 7 multidrug resistant *intl2* positive *A. baumannii* isolates, 6 of which were found to harbour the Tn7::In2-8 element. Our results demonstrate the unusually high distribution of Tn7::In2-8 among different *A. baumannii* clones from Chile, suggesting a particular behavior of these elements at geographical level.

Key words: Class 2 integron, *Acinetobacter baumannii*, antibiotic resistance

RESUMEN

Dispersión de Tn7::In2-8 en aislamientos multiresistentes de *Acinetobacter baumannii* de Chile. *Acinetobacter baumannii*, patógeno de importancia clínica en el ámbito hospitalario, es reconocido como un microorganismo que posee la capacidad de evolucionar rápidamente hacia la multiresistencia. Estudios previos efectuados en nuestro laboratorio han demostrado la alta dispersión de los integrones de clase 2 en aislamientos de esta especie. En el presente trabajo se analizaron 7 aislamientos de *Acinetobacter baumannii* multiresistentes portadores de la integrasa de clase 2, 6 de los cuales portaban el inusual arreglo Tn7::In2-8. Nuestros resultados muestran una elevada frecuencia de dispersión del elemento Tn7::In2-8 en diferentes clones circulantes en Chile, lo que sugiere un comportamiento geográfico particular.

Palabras clave: Integron de clase 2, *Acinetobacter baumannii*, resistencia antibiótica

Acinetobacter baumannii (Ab) is considered a serious threat in the hospital environment over the world (2, 5). Recently, it has also emerged as an important pathogen among soldiers returning from the Iraq and Afghanistan war zones (5). Furthermore, some Ab strains have become resistant to almost all currently available antibacterial agents through multifactorial combinatory antimicrobial resistance mechanisms (5). Class 1 and 2 integrons previously found in this species (3, 7) are elements that contain the genetic determinants of the components of a site-specific recombination system which recognizes and captures antimicrobial gene cassettes contributing to the multidrug resistant phenotype (4).

In the GenBank, in contrast with the large amount of cassette rearrangements reported for class 1 integrons, there are only 11 arrays of class 2 integrons described as having a different architecture from the one widespread over the world, embedded in the Tn7 transposon with

antimicrobial resistance gene cassettes within the variable region (8). A special characteristic of most type 2 integrase genes described so far is that they are not functional due to the presence of an internal stop codon (7), which most commonly explains the few cassette arrays in this class.

The aim of this study was to characterize 7 *intl2* positive *A. baumannii* multidrug resistant clinical isolates from 4 distinct regions of Chile, that were randomly chosen from a collection of ninety (n = 90) multidrug resistant *A. baumannii* *intl2* positive isolates recovered from a total of two hundred and twelve (n = 212) Ab strains from 24 different hospitals (Table 1) during the period 1990-2006. Total DNA was extracted and subjected to PCR amplification with specific primers (7). To detect the inserted gene cassettes, the class 2 integron variable region was amplified, and PCR cartography was also done as previously described (7). Both DNA strands were

Table 1. Description of the clinical isolates used in the study.

Isolate	City	Specimen	Year	Hospital	Genotype ^a	Resistant pattern ^b	Gene cassettes in the variable region
Ab1	Concepción	Burn infection	1997	H1 ^c	A	AMK-NEO-GEN-TOB-SXT	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab2	Valparaíso	Blood culture	1991	H2 ^c	A	AMK-NEO-GEN-TOB-SXT-CAZ-CIP	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab3	Concepción	Bronchial Secretion	1997	H1 ^c	A	AMK-NEO-GEN-TOB-SXT	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab4	Santiago de Chile	Femur liquid	1991	H3 ^c	A	AMK-NEO-GEN-TOB-SXT-CAZ-CIP	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab5	Valparaíso	Surgical wound	1990	H3 ^c	B	AMK-NEO-GEN-TOB-SXT-CAZ-CIP	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab6	Concepción	Bronchoalveolar lavage	1998	H1 ^c	A	AMK-NEO-GEN-TOB-SXT-CIP	<i>sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>
Ab7	Talcahuano	Blood culture	2006	H4 ^c	C	AMK-GEN-TOB-SXT-CAZ-CIP-FEP	<i>dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA</i>

^a Genotype was determined by repetitive extragenic palindromic DNA sequence-based PCR (REP-PCR). The different clones were named as A, B and C respectively.

^b AMK: amikacin; FEP: cefepime; CAZ: ceftazidime; CIP: ciprofloxacin; GEN: gentamicin; NEO: neomycin; TOB: tobramycin; SXT: trimethoprim-sulfamethoxazole.

^c H1, H2, H3 and H4 correspond to the hospitals where the isolates were obtained.

sequenced, using an ABIPrism 3100 BioAnalyzer equipment. The sequences were analyzed with the Blast V2.0 software (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Surprisingly, the class 2 integron called Tn7::In2-8 (7), harbouring an unusual array of gene cassettes within the variable region *sat2-aadB-catB2(ΔattI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA* was found in 6 out of the 7 isolates. Sequence analysis of the gene cassettes and of unusual cassettes found in the Tn7::In2-8 revealed that all of them are identical to the previously described array (7). The other strain harbored the typically found array in class 2 integrons embedded in the Tn7 transposon. We have previously described the presence of integron Tn7::In2-8 in three out of 106 *A. baumannii* clinical samples harbouring class 2 integrons (7, 8). The AB1 isolate was recovered in 1994, and isolates AB28 and AB29, in Argentina in the year 2003. Two of the isolates belonged to the same clone, and AB1 belonged to clone IV that was found to have epidemic behaviour in 5 hospitals from Buenos Aires City (1). By repetitive extragenic palindromic DNA sequence-based PCR (REP-PCR) and according to the cut-off level previously determined (6), the 7 isolates from this study exhibited 3 distinguishable clones, all different from those detected in

Argentina (Table 1). One possibility to explain the high dispersion of class 2 integrons in our *A. baumannii* isolates, considering these and previous results from our laboratory, is that the different clones of *A. baumannii* dispersed in the Southern Cone of Latin America are more likely or tend to carry class 2 integrons in their genome (7).

The five transposition genes of the Tn7 transposon were also found in all 7 isolates bearing 100% identity to the transposition genes of accession number X17693. In addition, we determined by PCR and sequence analysis that all Tn7::In2-8 were inserted in the specific *attTn7Ab* site in the chromosome of the *A. baumannii* isolates (3), and that all type 2 integrase genes harboured the internal stop codon.

Our results agree with the low amount of cassette arrays in class 2 integrons found over the world. In addition, the putative mechanisms involved in the architecture of gene cassettes in In2-8, homologous recombination and/or illegitimate integrase intermolecular recombination, suggest that recombination mediated by integron integrases is not only playing an important role for array evolution in class 2 integrons. This study also evidences an unusual high distribution of the Tn7::In2-8 element among different *A. baumannii* clones in 3 different cities

of Chile (Valparaíso, Santiago de Chile and Concepción), when compared to the low distribution of class 2 integrons in *A. baumannii* over the world (8). We have also previously reported the presence of this element in 3 out of 106 *intI2* positive isolates of *A. baumannii* from Argentina (7, 8). This result could correspond to the regional dispersion of Tn7::In2-8 among *A. baumannii* isolates from Chile and Argentina.

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