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Novel Rearrangement of a Class 2 Integron in Two Non-Epidemiologically Related Isolates of *Acinetobacter baumannii*

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Tn*7*::In2-8 contains *sat2-aadB-catB2*(Δ*attC*)*-dfrA1-sat2-aadA1-orfX* in the variable region of a class 2 integron embedded in the Tn*7*-like transposon. This novel transposon was inserted in its preferred site downstream of the *glms* gene in *Acinetobacter baumannii*. Acquisition of the pseudocassette *catB2* could have arisen by a secondary-site integrase-mediated intermolecular recombination event.

Integrons are elements that contain the genetic determinants of the components of a site-specific recombination system that recognizes and captures mobile gene cassettes (8).

The different integrons are classified according to their respective integrase genes. Class 2 integrons are embedded in the Tn*7* family of transposons. Tn*7* includes a defective integron consisting of the gene cassettes *dfrA1-sat2-aadA1-orfX* in its variable region (9, 16). In the GenBank, there are only six class 2 integrons described so far, Tn*1825* (20), Tn*4132* (24), Tn*7*::ISI-*ere-A* (2), a class 2 integron with GenBank accession no. AB161461 (1), a recently described class 2 integron in *Burkholderia cenocepacia* (DQ082896), and Tn*7* (9). The class 2 integron integrase, which is less than 50% homologous to the IntI1 integrase, is not functional due to the presence of an internal stop codon (9). Class 2 integrons have been reported in *Acinetobacter* spp. isolates throughout the world (7, 14, 19).

Here, we describe a novel rearrangement of a class 2 integron, Tn*7*::In2-8, in three isolates of *Acinetobacter baumannii*, with new cassettes in the variable region of a class 2 integron.

A. baumannii AB28 was isolated in 2003 in a public hospital (H1) of Buenos Aires from the tracheal aspiration of a patient in the coronary unit (intensive care unit). Susceptibility tests were performed using a disk diffusion method, NCCLS, 2003 (13). AB28 was resistant to gentamicin, amikacin, ceftazidime, and ciprofloxacin, and it had an intermediate susceptibility to imipenem and meropenem according to the breakpoint of the NCCLS method (13). The pulsed-field gel electrophoresis analysis performed with the AB28 isolate showed that it belonged to clone VI (data not shown).

Total DNA of AB28 was extracted as previously described (18) and subjected to PCR amplifications with specific primers for the class 2 integrase gene (15). To detect the inserted gene cassettes, the class 2 integron variable region was amplified with primers 125′CS and 23′CS (Table 1), which annealed in the flanking sequences of this region (*intI2* and *tnsE* genes). An amplicon product of approximately 6,400 bp was obtained, which was bigger than expected for Tn*7* (4,383 bp). In order to characterize this integron, PCR cartography (Table 1) and sequence analysis were performed using an Applied Biosystems 373 sequencer. The sequences were analyzed with Genetics Computer Group software (Wisconsin package, version 10.3).

The analysis revealed the presence of a duplication of the *sat2* cassette, which confers resistance to streptothricin, and the presence of the following two new resistant determinants for a class 2 integron: the *aadB* gene cassette (conferring resistance to gentamicin, kanamycin, and tobramycin) and the *catB2*(Δ*attC*) pseudocassette (conferring resistance to chloramphenicol). The sequence analysis established that downstream of the stop codon of the *catB2* gene there is a 259-bp region with 100% homology to the *attI2* class 2 integron recombination site replacing the typical *attC* site. Subsequent to this region, the same cassettes of the variable region of Tn*7* were found, resulting in the novel integron structure *sat2-aadB-catB2*(Δ*attC*)*-dfrA1-sat2-aadA1-orfX* (Fig. 1).

Sequence analysis of this region revealed that a nonelucidated mechanism might be involved in the acquisition of the pseudocassette *catB2* in the variable region of Tn*7*::In2-8. On one hand, the site where the excision in the *attI2* was done, TT′TTA (the prime denotes the recombination point), does not belong either to the core sites GTTRRRY of *attI1* and *attC*, which are the sites that IntI1 preferentially recognizes, or to the secondary sites conformed by the consensus GWTMW (5). Previously, this consensus sequence (GWTMW) has been found in the fused *ant*(3″)*-Ia*(Δ*attC*)*-bla*$_{OXA-9}$ cassette in Tn*1331* (21). On the other hand, the site where the cleavage in the *attC* of the *catB2* occurred, GCCTAA′C, corresponds to the inverse core site of the *attC*, complementary to GTTR RRY. In fact, cassettes are excised and then integrated at the preferential core site GTTRRRY, with the crossing over between the G and the first T (17) as different from the secondary site. An illegitimate integrase-mediated intermolecular recombination event involving similar sequences has been suggested for the creation of the fused *ant*(3″)*-Ih-*(Δ*attC*)*-aac*(6′)*-IId* in SCH909 (3). Therefore, the acquisition of the pseudocassette *catB2* in Tn*7*::In2-8 could have been caused by an analogous event involving the inverted core site of the *attC* site of *catB2*

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TABLE 1. Oligonucleotide sequences used in this study

Primer name	Sequence (5′-3′)	Reference or source
125′CS	TTTTTGTGCTGCCATATCCGTG	This work
23′CS	TGGGCTGAGAGAGTGGT	This work
satF	TGAGCAGGTGGCGGAAAC	This work
satR	TCATCCTGTGCTCCCGAG	This work
dhfR1	AGCTGTTCACCTTTGGC	10
dhfR1R	CCTGAAATCCCCAGCAA	This work
aadA1	TCGATGACGCCAACTAC	10
catB2R	AACAAAGAAAAGGCATTGC	This work
catB2F	CTGACTGAGCAGGTGAAG	This work
aadBF	GTAACACGCAAGCACGATGA	This work
aadBR	GCCTGTAGGACTCTATGTGC	This work
glmsF	GGCGGTCAGTTGTATGTCTT	6
Tn75′R	GACTCGTCCCGTCTTATGAG	6
tnsER	TCGATTTGCTGCTTTTGATG	This work
tnsEFF	TTGCTCTCTAACCACTCT	This work
tnsDR	CCGTCTAATTTGATAATCTTC	This work
tnsDF	GGGATTGTTAGTCCTAAGC	This work
tnsCR	GCTATCCCAGTCGCTGGG	This work
tnsCF	GTTTATCGTGATACGGGGG	This work
tnsBR	GAGCAAGCATTTACAAAAGC	This work
tnsBF	CATGTGGTCCAAGAACATAAG	This work
tnsAR	GCTAACAGTACAAGAAGTTCC	This work
tnsAF	CTCCATATTCACTACTTGGCT	This work

(CTAA′CAAT) and the end of an *attI*2 site (GTTTT′TTACG) preceding the *dfrA1* cassette of Tn*7*.

Using different primers designed for the 3′CS region where the transposition genes reside (Table 1), we carried out PCR and sequence analysis. We found the five transposition genes described before (6, 22) with 100% homology to the transposition genes of accession number X17693 (4).

We have also confirmed that this new rearrangement was inserted in the chromosome of *A. baumannii* using the specific primers described by Gay et al. (6). This set of primers amplifies the *att*Tn*7* site of *Escherichia coli* and a region of the Tn*7* transposon. By PCR and sequence analysis, we found that Tn*7*::In2-8 was inserted downstream of the *glms* housekeeping gene (11, 12, 23).

We also proceeded to test if this new rearrangement could be found in our collection of multiresistant *A. baumannii* isolates. For that purpose, we performed PCRs with the primers Inti2R and CatB2R in 65 clinical isolates of our collection, and only 2 isolates (AB29 and AB1) were positive for this reaction. Moreover, PCR cartography showed the same novel rearrangement observed in AB28. One of the isolates (AB29) corresponds to the same hospital (H1), the same year (2003), and the same clone (data not shown). It came from a patient's blood from the clinical medical area (not an intensive care unit). Possibly, clonal spread between these two different patients may have occurred since it is the same clone with the same resistance pattern and both isolates carried this new class 2 integron. The other isolate (AB1) came from another hospital (H2) and was collected from a patient's blood in 1994, and its pulsed-field gel electrophoresis analysis showed that it belonged to clone IV (data not shown). Susceptibility tests revealed that this isolate (AB1) was resistant to only ceftazidime, aztreonam, and piperacillin.

AB29 and AB1 were also localized in the chromosome downstream of the *glms* housekeeping gene, as previously described for the transposon Tn*7* (12).

This study reports a new rearrangement, Tn*7*::In2-8, found in two non-epidemiologically related multiresistant *A. baumannii* clones containing *aadB* and *catB2*, never described in a class 2 integron context before. The acquisition of new determinants of resistance in the variable region of integrons mediated by an illegitimate integrase intermolecular recombination event is likely to play an important role not only in the evolution of bacterial and plasmid genomes but also in the creation of novel rearrangements in the variable region of integrons that frequently contribute to the multiresistance challenge.

Nucleotide sequence accession number. The sequence of In2-8 has been submitted to GenBank under accession number DQ176450.

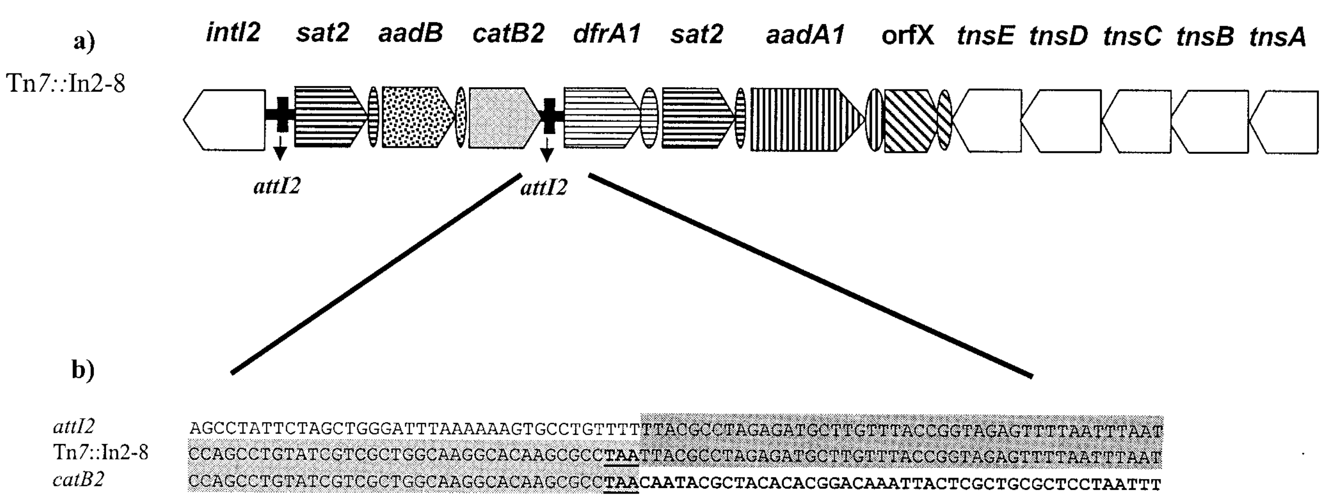

FIG. 1. Schematic representation of the structure of Tn*7*::In2-8. a) The *attI2* site is indicated as shown, and the ovals represent the *attC* sites of the gene cassettes. All *intI2* genes sequenced so far reveal an internal stop codon. b) Nucleotide sequence of *catB2* pseudocassette and comparison of this sequence with the *attC* site of the *catB2* cassette (accession number AJ487034) and the 5′CS region of the class 2 integron (accession number NC_002525).

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REFERENCES

1. **Ahmed, A. M., H. Nakano, and T. Shimamoto.** 2005. Molecular characterization of integrons in non-typhoid *Salmonella* serovars isolated in Japan: description of an unusual class 2 integron. J. Antimicrob. Chemother. **55:** 371–374.
2. **Biskri, L., and D. Mazel.** 2003. Erythromycin esterase gene *ere*(*A*) is located in a functional gene cassette in an unusual class 2 integron. Antimicrob. Agents Chemother. **47:**3326–3331.
3. **Centron, D., and P. H. Roy.** 2002. Presence of a group II intron in a multiresistant *Serratia marcescens* strain that harbors three integrons and a novel gene fusion. Antimicrob. Agents Chemother. **46:**1402–1409.
4. **Flores, C., M. I. Qadri, and C. Lichtenstein.** 1990. DNA sequence analysis of five genes, *tnsA*, *B*, *C*, *D* and *E*, required for Tn7 transposition. Nucleic Acids Res. **18:**901–911.
5. **Francia, M. V., F. de la Cruz, and J. M. Garcia Lobo.** 1993. Secondary-sites for integration mediated by the Tn*21* integrase. Mol. Microbiol. **10:**823–828.
6. **Gay, N. J., V. L. Tybulewicz, and J. E. Walker.** 1986. Insertion of transposon Tn7 into the *Escherichia coli glmS* transcriptional terminator. Biochem. J. **234:**111–117.
7. **Gonzalez, G., K. Sossa, S. Mella, R. Zemelman, H. Bello, and M. Domiguez.** 1998. Presence of integrons in isolates of different biotypes of *Acinetobacter baumannii* from Chilean hospitals. FEMS Microbiol. Lett. **161:**125–128.
8. **Hall, R. M., and C. M. Collis.** 1995. Mobile gene cassettes and integron: capture and spread of gene by site-specific recombination. Mol. Microbiol. **15:**593–600.
9. **Hansson, K., L. Sundstrom, A. Pelletier, and P. Roy** 2002. IntI2 integron integrase in Tn*7*. J. Bacteriol. **184:**1712–1721.
10. **Lévesque, C., L. Piché, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
11. **Lichtenstein, C., and S. Brenner.** 1982. Unique insertion site of Tn*7* transposition into the *E. coli* chromosome. Nature **297:**601–603.
12. **McKown, R. L., K. A. Orle, T. Chen, and N. Craig.** 1988. Sequence requirements of *Escherichia coli att*Tn*7*, a specific site of transposon Tn*7* insertion. J. Bacteriol. **170:**352–358.
13. **NCCLS.** 2003. Methods for diffusion antimicrobial susceptibility test for bacteria that grow aerobically. M2–A8. NCCLS, Wayne, Pa.
14. **Oh, J. Y., K. S. Kim, Y. W. Jeong, J. W. Cho, J. C. Park, and J. C. Lee.** 2002. Epidemiological typing and prevalence of integrons in multiresistant *Acinetobacter* strains. APMIS **110:**247–252.
15. **Orman, B. E., S. A. Piñeiro, S. Arduino, M. Galas, R. Melano, M. I. Caffer, D. O. Sordelli, and D. Centrón.** 2002. Evolution of multiresistance in non-typhoid *Salmonella* serovars from 1984 to 1998 in Argentina. Antimicrob. Agents Chemother. **46:**3963–3970.
16. **Radstrom, P., O. Skold, G. Swedberg, J. Flensburg, P. H. Roy, and L. Sundstrom.** 1994. Transposon Tn*5090* of plasmid R751, which carries an integron, is related to Tn*7*, Mu, and the retroelements. J. Bacteriol. **176:** 3257–3268.
17. **Recchia, G. D., H. W. Stokes, and R. M. Hall.** 1994. Characterization of specific and secondary recombination sites recognized by the integron DNA integrase. Nucleic Acids Res. **22:**2071–2078.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Seward, R. J., and K. J. Towner.** 2002. Detection of integrons in world-wide nosocomial isolates of *Acinetobacter* spp. Clin. Microbiol. Infect. **5:**308–318.
20. **Tietze, E., J. Brevet, H. Tschape, and W. Voigt.** 1988. Cloning and preliminary characterization of the streptothricin resistance determinants of the transposons Tn*1825* and Tn*1826*. J. Basic Microbiol. **28:**129–136.
21. **Tolmasky, M. E., and J. H. Crosa.** 1993. Genetic organization of antibiotic resistance genes (*aac*(*6′*)*-Ib*, *aadA*, and *oxa9*) in the multiresistance transposon Tn*1331*. Plasmid **29:**31–40.
22. **Waddell, C. S., and N. L. Craig.** 1988. Tn*7* transposition: two transposition pathways directed by five Tn*7*-encoded genes. Genes Dev. **2:**137–149.
23. **Walker, J., N. Gay, M. Saraste, and A. Eberle.** 1984. DNA sequence around the *Escherichia coli unc* operon. Biochem. J. **224:**799–815.
24. **Young, H., M. J. Qumsich, and M. L. Mcintosh.** 1994. Nucleotide sequence and genetic analysis of the type Ib trimethoprim-resistant, Tn*4132*-encoded dihydrofolate reductase. J. Antimicrob. Chemother. **34:**715–725.