ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5294–5296 Vol. 53, No. 12
0066-4804/09/$12.00 doi:10.1128/AAC.00687-09

Tn*6060*, a Transposon from a Genomic Island in a *Pseudomonas aeruginosa* Clinical Isolate That Includes Two Class 1 Integrons▽

Piklu Roy Chowdhury,[1] John Merlino,[2] Maurizio Labbate,[1] Elaine Y.-L. Cheong,[2] Thomas Gottlieb,[2] and H. W. Stokes[1]*

Institute for the Biotechnology of Infectious Diseases, Department of Medical and Molecular Biosciences, University of Technology, Sydney, P.O. Box 123 Broadway, NSW 2007, Australia,[1] and Department of Microbiology and Infectious Diseases, Concord Hospital, Hospital Road, Concord 2139, NSW, Australia[2]

Received 19 May 2009/Returned for modification 4 July 2009/Accepted 5 September 2009

A 25,441-bp transposon was recovered from a *Pseudomonas aeruginosa* clinical isolate. While the transposition module was >99% identical to sequence of Tn*1403*, the element had been subject to rearrangements, with two In70.2-like class 1 integrons inserted into it in an unusual "tail-to-tail" configuration. One cassette array was the same as that in In70.2; however, the second was different, generating a transposon that collectively includes six resistance cassettes.

Infections caused by multidrug-resistant gram-negative bacteria are a continuing and growing problem (3). A significant contributor within this cohort is *Pseudomonas aeruginosa*, a species that shows a great diversity of resistance profiles, with resistance to ß-lactam antibiotics being most notable (16). In recent years, a number of multidrug-resistant *P. aeruginosa* strains have been isolated that contain class 1 integrons with novel resistance cassettes, including several that contain determinants conferring resistance to β-lactams (5, 9, 16). Here we report the recovery of a VIM-1 metallo-β-lactamase-producing *P. aeruginosa* clinical isolate with a novel transposon that included two class 1 integrons.

Repeated urinary specimens from a patient in Sydney, Australia, with a long-standing, treated prosthetic knee infection yielded a highly multidrug-resistant *P. aeruginosa* strain. The isolate was carbapenem resistant and underwent further analysis for the presence of a metallo-β-lactamase. When noted to be VIM-1 positive, the isolate, recovered in 2008 (8) and here designated strain 37308, was referred and analyzed in detail. Initial screening by PCR with the class 1 integron-specific primers HS915 and HS916 (7) suggested the presence of such an element. To investigate the genetic context of this class 1 integron, a fosmid genomic library was constructed (1). *intI1*-positive clones were identified by PCR with the primers HS915 and HS916, and one such clone, F32, was completely sequenced. The cloned region was 43,062 bp in length. The strain was found to contain two closely linked class 1 integrons, both of which were contained within a transposon, here designated Tn*6060*.

Tn*6060* was inserted within a previously identified genomic island, which, in the *P. aeruginosa* strain PACS171b (recovered from a cystic fibrosis patient [4]), is 36,840 bp long (accession number EU595750). The genomic island is not present in the *P. aeruginosa* strain PAO1. The equivalent island region in clone F32 is very similar (99% identity) to that of PACS171b over the length of available sequence (17,616 bp) in the former, with the notable exception of two deletions of DNA in the F32 clone of 113 bp and 2,859 bp in length. Insertion of Tn*6060* generated 5-bp flanking direct repeats of the genomic island sequence, characteristic of transposition of Tn*21*-like transposons. The duplicated target comprised bases 9304 to 9308 in relation to the PACS171b genomic island sequence (Fig. 1A).

The structure of Tn*6060* is shown in Fig. 1B. The transposition module extending from the 38-bp inverted repeat to the point of insertion of the right-hand integron (Fig. 1B) displays only a 2-bp difference from sequence of Tn*1403* (15). The point of insertion of the large-array integron (comprising the cassettes *aadA1*, *aphA15*, *aacA4*, and bla_{VIM1}) with reference to IRi of that integron is the same as that for In28 in Tn*1403*. However, the integron itself is very different from In28 and instead has a structure most closely related to that of In70.2 (14), with a copy of IS*Pa7* inserted in the 5′ conserved segment (5′-CS) at the same point where it appears in In70.2. In addition, this integron, while comprising a defective transposition module, lacks the insertion elements normally associated with the In4 family (including In28) or In5 family of class 1 integrons (10, 12, 15). Beyond the IRt inverted repeat is a 547-bp region identical to sequence within the *urf2* gene, which is part of the Tn*21*-like mercury resistance transposons (11). The sequence abuts the IRi end of a second class 1 integron with a different, smaller cassette array, *aacC3-cmlA5*. This second integron is identical to the first with the exception of a different cassette array. Sequence outside the 5′-CS of this small-array integron (i.e., to the left of IRi in Fig. 1B) is identical to that adjacent to IRi of In70.2 in the sequence under GenBank accession no. AJ581664 up to the point where the latter sequence is available. The region between IRi of the small-array

* Corresponding author. Mailing address: Institute for the Biotechnology of Infectious Diseases, Department of Medical and Molecular Biosciences, University of Technology, Sydney, P.O. Box 123, Broadway, NSW 2007, Australia. Phone: (612) 9514 2232. Fax: (612) 9514 8206. E-mail: hatch.stokes@uts.edu.au.

▽ Published ahead of print on 14 September 2009.

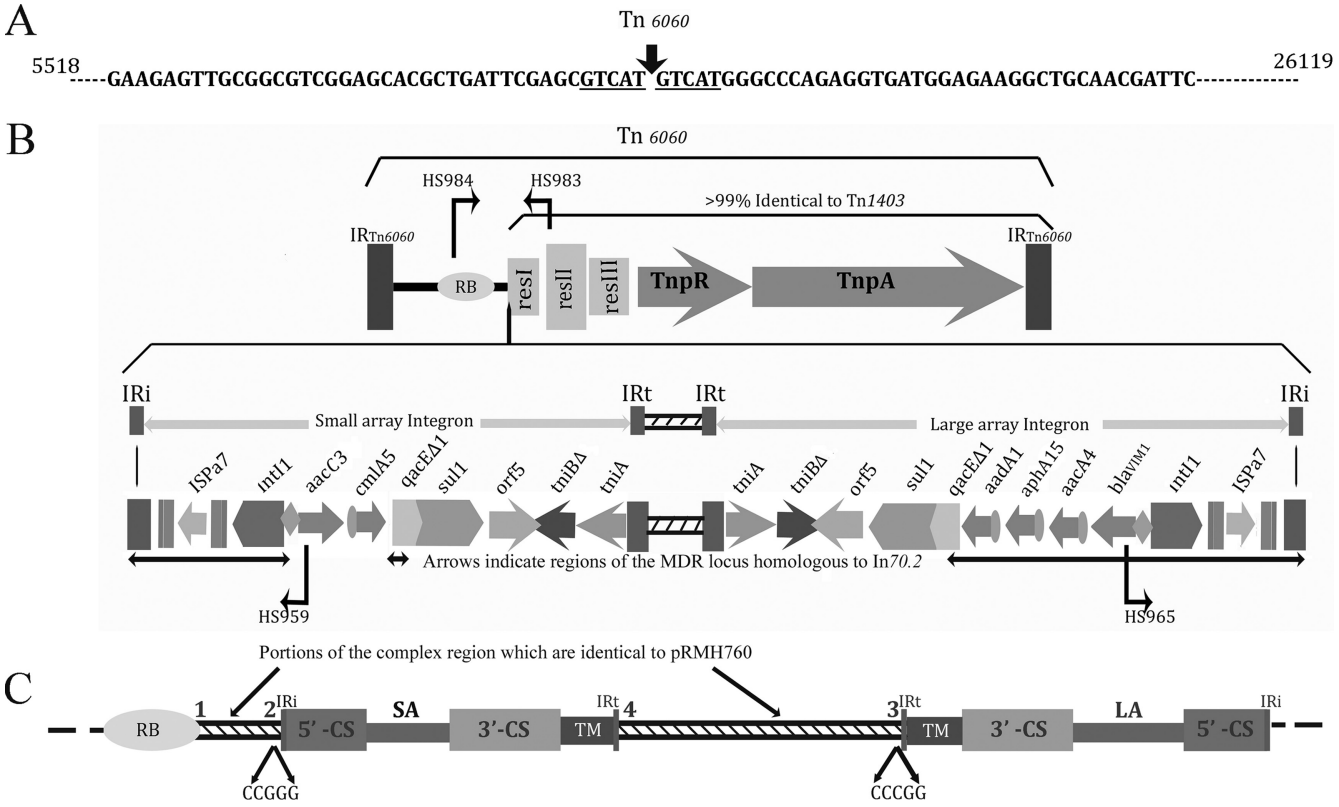

FIG. 1. Tn*6060* and its location in the *Pseudomonas aeruginosa* strain 171b genome fragment cloned as fosmid 32. (A) Sequence shown is the immediate region of the pathogenicity island as identified in PACS171b (accession number EU595750) that surrounds the transposon. Flanking numbers indicate the boundaries of the pathogenicity island referenced to the same database entry. Underlined sequence is the direct repeat generated by transposon insertion. (B) Detailed structure of transposon Tn*6060*. IRi and IRt represent the inverted repeats found at the integron end and transposition module end, respectively, of class 1 integrons/transposons. The hatched region in the middle represents a region of identity to pRMH760. Bent arrows identify PCR primers referred to in the text. Other key integron features are as defined previously (12, 15). Resistance cassettes are as defined previously (5, 9). (C) Diagrammatic representation of the two integrons, highlighting the relationship between the two integrons, sequence common to pRMH760, and direct transposon repeats. Hatched double lines indicate regions of identity to pRMH760 (accession number AY123253.3). The region labeled 3-4, when inverted, is sequence contiguous with region 1-2 in pRMH760. The pair of 5-base sequences indicated by arrows become direct repeats flanking IRi/IRt in the inverted sequence (see the text). RB, resolvase binding site; SA, small-array integron; LA, large-array integron; TM, Tn*402*-like transposition module.

integron and the end of the Tn*1403*-like inverted repeat is 286 bases in length.

None of this sequence is related to that of Tn*1403*, including the Tn*6060* inverted repeat at this end, suggesting that this region is derived from another, yet to be identified transposon.

There are precedents for replicons/transposons containing two integrons, and these probably arise by homologous recombination between conserved regions of integrons (2, 11) that then generates a tandem "head-to-tail" array in the product. In contrast, the two integrons here are arranged "tail to tail" (Fig. 1B) and are therefore present as large inverted repeats across the extensive sequences in common. In clone F32, this has resulted in an inversion between the two integrons at some point prior to sequencing. This is evident from the fact that the 5-bp sequence (CCGGG), immediately abutting IRi, of the small-array integron is the reverse complement of the sequence (CCCGG) located next to IRt of the large-array integron (Fig. 1C). This 5-bp duplication would have arisen from the transposition event that resulted in one of the class 1 integron/Tn*402*-like transposons in Tn*6060* being inserted into the pRMH760-related sequence. As also shown in Fig. 1C, alignment of the indicated sequences with the pRMH760 (11) sequence also implies an inversion event.

To assess the orientation of the two integrons within the *P. aeruginosa* genome in strain 37308, we carried out PCR using four primers in pairwise reactions. One primer in each pair (HS983 [5′ TGGCAAGCATGTGTCAAAGAATGTG 3′] and HS984 [5′ CCGGCCTGACGCCTAGTTA 3′]) targets sequences beyond the two IRi sites, and the second in each pair targets sequences unique to the large-array (HS965 [5′ CTGT CGGATACTCACCACTC 3′]) and small-array (HS959 [5′ GGTAACTGTCGCTTGGTTGAG 3′]) integrons (Fig. 1B). In the orientation found in F32 (Fig. 1B), only two of the four PCRs will generate a product. These are HS983 with HS965 and HS984 with HS959. If the two integrons invert, the other two primer pair combinations will generate a product. Interestingly, all four PCRs carried out with DNA extracted from an overnight growth culture of strain 37308 generated a product, although the two pairs targeting the orientation opposite to that shown in Fig. 1 were significantly brighter (data not shown), implying that this second tail-to-tail orientation was the "parent" in this clone. In any event, it is clear that this

region inverts at a high rate, particularly since this PCR method will detect only inversions involving DNA that is homologous between the respective primer pairs. This region between the primers constitutes only a small fraction of the DNA common to the two integrons.

We are unable to offer a precise genealogy for the creation of Tn*6060*. A number of events must have been involved, however, driven by both homologous recombination and site-specific resolution. One early event presumably involved homologous recombination between two class 1 integrons at different locations or between the 5′- and 3′-CS of two integrons, since the insertion points of the two integrons are different. Also, the right-hand integron (Fig. 1C) derived from the In28-like parent does not possess Tn*1403*-like sequence beyond IRt, even taking into account the inversion. Thus, the IRi end is located in the same position as In28 is in Tn*1403*, but the IRt end is not. Additionally, it is significant that both integrons have an identical class 1-specific 5′-CS with respect to the presence of ISPa*7* and 3′-CS. Since it is unlikely that the integron that is analogous to In28 with respect to the insertion point in the transposition module had an In70.2-like backbone, we hypothesize that this integron's 5′-CS was derived from the second integron by a gene conversion-like event after the two came together.

One of the difficulties in describing likely ancestry is the fact that while both cassette arrays have been described previously, contextual information is limited in previously published reports. The large array, which includes VIM-1, is present in In70.2, and this has the same backbone over the available sequence (14). However, the published sequence extends only to the *resI* site just beyond IRi and stops before the end of the 3′-CS and the IRt boundary. Given that In70.2-like elements are common in *P. aeruginosa* isolates in Europe, it would be interesting to determine if this integron backbone is being disseminated by Tn*6060*. We found that Tn*6060* is transposable to a conjugative plasmid (mean frequency, 9.4×10^{-3}/donor over three independent assays), so this is possible. Despite the fact that the two arrays have been seen individually in *P. aeruginosa* clinical isolates (9, 14), they have not till now been reported together. However, this may be due partly to the experimental design of many studies, in which integron arrays are detected via primers that flank conserved regions of class 1 integrons (6). Where multiple arrays are present, biases in conventional PCR may preclude detectable amplification of larger arrays at the expense of smaller ones. Also, the point has been made previously (11, 15) that a variety of genetic elements and processes contribute to the spread of resistance and that detailed examination of the context in which such elements as integrons are located can be particularly insightful in comparing and understanding multidrug-resistant isolates (7, 13). More systematic and detailed sequence analyses may therefore shed light on the mechanisms by which integrons and their arrays move through pathogens.

Nucleotide sequence accession number. The sequence of fosmid clone F32, which includes Tn*6060* and surrounding sequence, has been submitted to GenBank under accession no. GQ161847.

This work was supported in part by the Institute for the Biotechnology of Infectious Diseases, University of Technology, Sydney, Australia.

REFERENCES

1. **Boucher, Y., C. L. Nesbo, M. J. Joss, A. Robinson, B. C. Mabbutt, M. R. Gillings, W. F. Doolittle, and H. W. Stokes.** 2006. Recovery and evolutionary analysis of complete integron gene cassette arrays from *Vibrio*. BMC Evol. Biol. **6:**3.
2. **Ceccarelli, D., A. M. Salvia, J. Sami, P. Cappuccinelli, and M. M. Colombo.** 2006. New cluster of plasmid-located class 1 integrons in *Vibrio cholerae* O1 and a *dfrA15* cassette-containing integron in *Vibrio parahaemolyticus* isolated in Angola. Antimicrob. Agents Chemother. **50:**2493–2499.
3. **Giske, C. G., D. L. Monnet, O. Cars, and Y. Carmeli.** 2008. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob. Agents Chemother. **52:**813–821.
4. **Hayden, H. S., W. Gillett, C. Saenphimmachak, R. Lim, Y. Zhou, M. A. Jacobs, J. Chang, L. Rohmer, D. A. D'Argenio, A. Palmieri, R. Levy, E. Haugen, G. K. Wong, M. J. Brittnacher, J. L. Burns, S. I. Miller, M. V. Olson, and R. Kaul.** 2008. Large-insert genome analysis technology detects structural variation in *Pseudomonas aeruginosa* clinical strains from cystic fibrosis patients. Genomics **91:**530–537.
5. **Lauretti, L., M. L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana, and G. M. Rossolini.** 1999. Cloning and characterization of bla_{VIM}, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob. Agents Chemother. **43:**1584–1590.
6. **Levesque, C., L. Piche, C. Larose, and P. H. Roy.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39:**185–191.
7. **Marquez, C., M. Labbate, C. Raymondo, J. Fernandez, A. M. Gestal, M. Holley, G. Borthagaray, and H. W. Stokes.** 2008. Urinary tract infections in a South American population: dynamic spread of class 1 integrons and multidrug resistance by homologous and site-specific recombination. J. Clin. Microbiol. **46:**3417–3425.
8. **Merlino, J., H. W. Stokes, E. Y.-L. Cheong, and T. Gottlieb.** 2009. First clinical case of locally-acquired carbapenem resistant VIM-1 metallo-β-lactamase seen in *Pseudomonas aeruginosa* in Australia. Med. J. Aust. **191:**289.
9. **Ozgumus, O. B., R. Caylan, I. Tosun, C. Sandalli, K. Aydin, and I. Koksal.** 2007. Molecular epidemiology of clinical *Pseudomonas aeruginosa* isolates carrying IMP-1 metallo-β-lactamase gene in a university hospital in Turkey. Microb. Drug Resist. **13:**191–198.
10. **Partridge, S. R., H. J. Brown, H. W. Stokes, and R. M. Hall.** 2001. Transposons Tn*1696* and Tn*21* and their integrons In4 and In2 have independent origins. Antimicrob. Agents Chemother. **45:**1263–1270.
11. **Partridge, S. R., and R. M. Hall.** 2004. Complex multiple antibiotic and mercury resistance region derived from the r-det of NR1 (R100). Antimicrob. Agents Chemother. **48:**4250–4255.
12. **Partridge, S. R., G. D. Recchia, H. W. Stokes, and R. M. Hall.** 2001. Family of class 1 integrons related to In4 from Tn*1696*. Antimicrob. Agents Chemother. **45:**3014–3020.
13. **Post, V., and R. M. Hall.** 2009. AbaR5, a large multiple-antibiotic resistance region found in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53:**2667–2671.
14. **Riccio, M. L., L. Pallecchi, J. D. Docquier, S. Cresti, M. R. Catania, L. Pagani, C. Lagatolla, G. Cornaglia, R. Fontana, and G. M. Rossolini.** 2005. Clonal relatedness and conserved integron structures in epidemiologically unrelated *Pseudomonas aeruginosa* strains producing the VIM-1 metallo-β-lactamase from different Italian hospitals. Antimicrob. Agents Chemother. **49:**104–110.
15. **Stokes, H. W., L. D. Elbourne, and R. M. Hall.** 2007. Tn*1403*, a multiple-antibiotic resistance transposon made up of three distinct transposons. Antimicrob. Agents Chemother. **51:**1827–1829.
16. **Weldhagen, G. F.** 2004. Integrons and β-lactamases—a novel perspective on resistance. Int. J. Antimicrob. Agents **23:**556–562.