Tn6167, an antibiotic resistance island in an Australian carbapenem-resistant Acinetobacter baumannii GC2, ST92 isolate

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Objectives: To determine the context and location of the blaOXA-23 carbapenem-resistance gene and the structure of the resistance island in the chromosomal comM gene in a representative Australian global clone 2 (GC2) Acinetobacter baumannii isolate.

Methods: Long-range PCR was used to link genes and determine the organization of the resistance island. PCR amplicons were sequenced, and bioinformatic analysis identified features. Multilocus sequence typing (MLST) was performed.

Results: The GC2 isolate A91 is sequence type (ST) ST92 (Oxford MLST scheme). It includes a 37 kb genomic resistance island, Tn6167, in the comM gene. At one end, Tn6167 carries Tn6022Δ1 interrupted by a novel insertion sequence, ISAba17. The sul2 (sulphonamide resistance) and strA-strB (streptomycin resistance) genes and tet(B) tetracycline resistance determinant are at the other end in the configuration ISAba1-sul2-CR2Δ-tetA(B)-tetR(B)-CR2-strB-strA with part of the tni end of a Tn6022-related transposon preceding them and an orf4 end following them. Transposon Tn2006 carrying blaOXA-23 was found in an 11 kb region located between Tn6022Δ1 and the other resistance genes. The 17.6 kb Tn6166 from the GC2 reference strain A320/ RUH134 can be derived from Tn6167 via a single deletion arising adjacent to Tn6022Δ1 and causing loss of a large central segment.

Conclusions: The transposons found in comM in the GC2 isolates A91 and A320 differ substantially from AbaR3-type islands, found predominantly in global clone 1 (GC1) isolates, in both resistance gene content and organization. However, the A. baumannii GC1 and GC2 clones have both acquired antibiotic resistance genes via their association with transposons that target comM.

Keywords: multiply antibiotic-resistant A. baumannii, global clone 2, resistance islands, carbapenem resistance, transposon Tn6167, Tn6166, Tn2006

Introduction

Transposons that appear to preferentially incorporate themselves into a specific location in the comM gene of Acinetobacter baumannii have emerged as important vehicles for the acquisition of antibiotic resistance genes. The AbaR3-type antibiotic resistance islands found in most of the global clone 1 (GC1) isolates examined to date (see Nigro and Hall1 for references) have a backbone, designated Tn6019,2,3 that is a member of this group. AbaR4,4 which carries the blaOXA-23 carbapenem resistance gene in transposon Tn2006,5 has a related but simpler backbone designated Tn6022,6 and, though AbaR4 was first found in another location,7 it was recently found in comM in a GC1 isolate.5 Tn6021 in comM in ATCC 17978 is closely related to Tn6022, and carries no antibiotic resistance genes.5,6 These transposons include five genes—tniA, tniB, tniC, tniD and tniE—that are shared by several more distant relatives, and are likely to be required for transposition or targeted transposition.5

Much less is known about the resistance regions in the global clone 2 (GC2) complex. The first GC2 isolate sequenced includes just one end of an AbaR3-type island next to a remnant of comM.7 However, more complete transposons with ends related to those of AbaR3 or Tn6022 have been found in comM in most of the GC2 isolates so far examined,3,4,8–10 and most of these do not share features such as the arsenate resistance region, Tn6018 and an interrupted uspA gene that are characteristic of Tn6019 and hence AbaR3-related islands.3,4,8 Recently we described a third type of resistance island found in the same position in comM in the reference GC2 isolate RUH134 (A320) that was isolated in 1982.1 Transposon Tn6166

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is 17.6 kb long and has a complex structure (Figure 1a). It includes the tet(B) tetracycline resistance determinant and the strAB streptomycin resistance genes, which are not usually found in AbaR3-type transposons, and a copy of Tn6022 with a 2.85 kb deletion in it.1

Five Australian carbapenem-resistant isolates from a 2005 outbreak at a Sydney hospital were among those that have been shown to belong to GC2 and carry a distinct type of island in the comM gene.3 All five carry the carbapenem resistance determinant blaOXA-23 adjacent to an upstream copy of ISAba1, as well as ISAba1 upstream of the chromosomal ampC gene.11 They also carry the acquired resistance genes blaTEM, sul2, strA and strB and the tet(B) resistance determinant.11,12 In addition, they all carried a class 1 integron,3,11,12 and hence fell into only one of the five groups found among Australian carbapenem-resistant GC2 isolates that have different aminoglycoside resistance determinants.12 As the allelic variations in the sequences near the boundaries of the transposon inserted within comM for one of these isolates (GenBank accession number GQ914990)3 were the same as those found in Tn6166,1 the structure of the resistance island in a representative of this group was further investigated. In addition, the blaOXA-23 gene is found in a number of different transposon contexts, and a recent study demonstrated that it is found in several different locations in GC2 isolates,2 indicating that it has entered the clone on more than one occasion. Hence, the context and location of the ISAba1-blaOXA-23 configuration in one of the Australian isolates was also investigated.

Materials and methods

Bacterial isolates

A. baumannii A91, A93, A94, A96, A97 are carbapenem-resistant, multiply antibiotic-resistant isolates belonging to the GC2 clone that were isolated in 2005 at a Sydney hospital and have been characterized previously.3,11,12 The sequence type (ST) of A91 was determined according to the multilocus sequence typing (MLST) scheme hosted at Oxford University (http://pubmlst.org/abaumannii/) using modifications described previously,6 and submitted to the site.

PCR amplification, DNA sequencing and sequence analysis

Genomic DNA was extracted as described previously.12 Conditions used to detect short and long PCR amplicons were described previously.6 Primer pairs used to map and locate Tn2006 and map the insertion in comM are listed in Table 1.

Fifteen Australian carbapenem-resistant isolates from a 2005 outbreak at a Sydney hospital were among those that have been shown to belong to GC2 and carry a distinct type of island in the comM gene.3 All five carry the carbapenem resistance determinant blaOXA-23 adjacent to an upstream copy of ISAba1, as well as ISAba1 upstream of the chromosomal ampC gene.11 They also carry the acquired resistance genes blaTEM, sul2, strA and strB and the tet(B) resistance determinant.11,12 In addition, they all carried a class 1 integron,3,11,12 and hence fell into only one of the five groups found among Australian carbapenem-resistant GC2 isolates that have different aminoglycoside resistance determinants.12 As the allelic variations in the sequences near the boundaries of the transposon inserted within comM for one of these isolates (GenBank accession number GQ914990)3 were the same as those found in Tn6166,1 the structure of the resistance island in a representative of this group was further investigated. In addition, the blaOXA-23 gene is found in a number of different transposon contexts, and a recent study demonstrated that it is found in several different locations in GC2 isolates,2 indicating that it has entered the clone on more than one occasion. Hence, the context and location of the ISAba1-blaOXA-23 configuration in one of the Australian isolates was also investigated.

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Figure 1. Structures of (a) Tn6166 and (b) Tn6167. Regions with different origins are indicated by lines of various types and thicknesses and IRs are shown as vertical lines. The genes and open reading frames and their extent are shown by labelled arrows below. The small mobile element CR2 is shown as an open box with the ori end indicated. ISs are shown as open boxes with their name inside. The location of the deletion in Tn6022 is indicated by a vertical arrow marked △. In (a), the origin of different segments is indicated above and the asterisk indicates that the tetA(B) gene is truncated.
as described previously. Features of the island in comM in strain 1656-213 (GenBank accession number CP001921) were analysed in the same way.

A 37609 bp sequence from A91 consisting of Tn6167 (37068 bp) flanked by part of comM has been submitted to GenBank under accession number JN968483. The sequence of the new IS was submitted to IS-Finder (http://www-is.biotoul.fr/) and assigned the designation ISAba17. The transposon number was assigned a number via the Tn number registry at http://www.ucl.ac.uk/eastman/tn/.

### Results

**Location of the blaOXA-23 gene**

Isolate A91 was shown to carry the blaOXA-23 carbapenem resistance gene in the transposon Tn2006, using primers described elsewhere and listed in Table 1. However, Tn2006 was not located in AbaR4. Four other locations for Tn2006 in GC2 isolates have been reported, but adjacent sequence is available for only one of them (GenBank accession number GQ861439). Using a primer in this sequence to link to the blaOXA-23 gene (RH737 and OXA-23R in Table 1), Tn2006 in A91 was shown to occupy the same location. Searches revealed that the region adjacent to Tn2006 was highly conserved in AbaR4.

### Table 1. Primer pairs used

<table>
<thead>
<tr>
<th>PCR</th>
<th>Primers</th>
<th>Sequence</th>
<th>Predicted size (bp)</th>
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<tr>
<td>CR2-sul2</td>
<td>RH53</td>
<td>GGCTCAAGCGTTTTCAAT</td>
<td>NA</td>
</tr>
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<td>RH603</td>
<td>ACTTCAATCCACACACCG</td>
<td>3430&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>arf7-Tn2006</td>
<td>RH737</td>
<td>TGTAATTGCACAAATCAGCA</td>
<td>2326</td>
</tr>
<tr>
<td></td>
<td>OXA-23R</td>
<td>TCACAACCAAATATAAGCTGA</td>
<td>2326</td>
</tr>
<tr>
<td>comM-tniBΔ</td>
<td>RH791</td>
<td>TGCTCAATGAGGTCAAGTTGAAGT</td>
<td>3119</td>
</tr>
<tr>
<td></td>
<td>RH909</td>
<td>GCGATTCAAAATATCGTGCA</td>
<td>3119</td>
</tr>
<tr>
<td>comM-orfA</td>
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<td>4632&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>RH592</td>
<td>AAGCTTATGCAAAATAGCTGA</td>
<td>7234&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>tniBΔ-tniEΔ</td>
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<td>GCGATAGTGAAAGGTCAAGAGAA</td>
<td>560&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
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<td>RH587</td>
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<td>4213</td>
</tr>
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<td>ISAba17</td>
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<td>CCGTCAATGATGTTTGTTTG</td>
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<td></td>
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<td>GGGCGGCAAAATATAAGCTGA</td>
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<tr>
<td>arf6-Tn2006</td>
<td>RH1302</td>
<td>CAAATCGGGAAGGTTCAAAAA</td>
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<td>OXA-23R</td>
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<td>oxa23likEF</td>
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<td>ISAba1-tniCb</td>
<td>RH1301</td>
<td>TGGCAATTTAAAGAAGGCGA</td>
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<td>RH1314</td>
<td>TCCCTCATAAACCAACAAACCA</td>
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<td>tniC-sul2</td>
<td>RH590&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>sul2R</td>
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<td>ISAba1-CR2</td>
<td>ISAba1B</td>
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<td>LECR2</td>
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<td>tetR(B)-strB</td>
<td>RH892</td>
<td>ACAGGCAATGAGCCTGTT</td>
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<tr>
<td></td>
<td>RH928</td>
<td>GCCAGCAAGCTCAGTAAA</td>
<td>5021</td>
</tr>
</tbody>
</table>

<sup>a</sup>NA indicates not applicable.
<sup>b</sup>852 bp when CR2Δ-tet(B) is not present.
<sup>c</sup>7482 bp in Tn6022, distinguished by digestion.
<sup>d</sup>3410 bp in Tn6022.
<sup>e</sup>This primer is specific for orf4.
<sup>f</sup>This primer is specific for orf4.

**GenBank accession number, and insertion sequence (IS) and transposon designations**

A 37609 bp sequence from A91 consisting of Tn6167 (37068 bp) flanked by part of comM has been submitted to GenBank under accession number JN968483. The sequence of the new IS was submitted to IS-Finder (http://www-is.biotoul.fr/) and assigned the designation ISAba17. The transposon number was assigned a number via the Tn number registry at http://www.ucl.ac.uk/eastman/tn/.
to Tn2006 was present in the genome of ATCC 17978 (GenBank accession number CP000521) and a second primer, RH1305, designed using the ATCC 17978 sequence, was used to show that, in A91, Tn2006 interrupted this segment and was flanked by a 9 bp duplication of the target. The sequence of Tn2006 (4805 bp) and the adjacent segments from A91 were identical to those found in GenBank accession number GQ861439 or CP000521. Using the same PCRs, Tn2006 was shown to be present and in the same position in the remaining four isolates (A93, A94, A96, A97) in this group.

**ST**

The ST for A91 was determined to be ST92 (1-3-3-2-2-7-3) in the Oxford MLST scheme, and all of the isolates studied previously that had Tn2006 in the position described above were also ST92 (reported as ST22), a designation that was subsequently changed to ST92; see http://pubmlst.org/abaumannii/ for ST designation changes).

**Tn6167, the transposon in comM**

Initial mapping of the transposon in comM in A91 was undertaken with primer pairs used for Tn6166.1 Tn6022Δ1, a transposon identical to Tn6022 except for a deletion of 2.85 kb that removes the tniD gene and part of the flanking tniB and tniE genes,1 was present at the left end, but was interrupted by an IS, ISAba17, as shown in Figure 1(b).

In A91, the right-hand end (as shown in Figure 1) of the transposon in comM was also identical to the corresponding segment of Tn6166. A 1869 bp segment that contains orf4b, a relative of orf4, and is 86% identical to the corresponding portion of Tn6022 was located adjacent to comM. The tet(B)-CR2-strA-strB configuration was found adjacent to this transposon remnant, as in Tn6166. However, whereas in Tn6166 the tet(B) determinant lies immediately adjacent to the internal inverted repeat (IR) of Tn6022Δ1 and the last 6 bp of the tetA gene are missing, in A91 these regions could not be linked. Instead, the sul2 gene, which was originally associated with the small mobile element CR2,14,15 was found separated from the complete tetA gene by a partial copy of CR2, consisting of the first 194 bp of the ori end. This arrangement suggests that a circular DNA molecule made up of the tet(B) determinant and the CR2 remnant may have entered a resistance cluster containing only the sul2-CR2 and strA-B regions via homologous recombination between the identical CR2 segments. The copy of ISAba1 upstream of the sul2 gene is in the position found previously in GenBank accession number AY823412.16 and in ATCC 17978,14 and this IS may contribute to expression of sul2.16

Tn2006 was localized to the additional intervening region. A later analysis revealed that the region next to Tn2006 was also present in an island in comM in the recently released complete genome of GC2 strain 1656-2 from South Korea13 (GenBank accession number CP001921). The transposon in comM in strain 1656-2 was found to be related to Tn6166, but larger, with an additional 6.2 kb found between Tn6022Δ1 and ISAba1 in 1656-2. This 6.2 kb segment is also present in ATCC 17978 and was also detected in A91 interrupted by Tn2006 (shown in the central line in Figure 1b). However, 1656-2 and ATCC 17978 did not carry Tn2006. The transposon in A91 was designated Tn6167, and the complete sequence was determined. Tn6167 is 37.6 kb long and its structure is shown in Figure 1(b).

**ISAba17**

ISAba17 is 2594 bp long, bounded by 21 bp IRs, and has created a direct duplication of 8 bp. ISAba17 was not detected in any entry in the GenBank non-redundant DNA database, which, as of 5 October 2011, included the complete genomes of four GC2 A. baumannii isolates. ISAba17 is a member of the IS66 family17,18 and encodes three polypeptides. Their closest relatives in the GenBank protein database, sharing 52 and 64% identity, respectively, are the predicted products of the tnpB and tnpC genes of ISPpu19 (GenBank accession number AB238971). The TnpA product is not clearly related to TnpA of ISPpu19. The TnpC proteins include the DDE motif characteristic of transposases.18

**Discussion**

Tn6167, found here in an ST92 (1-3-3-2-2-7-3) isolate, and Tn6166, found previously in an ST98 (1-12-3-2-2-3-3) isolate,1 are representative of a type of genomic resistance island that differs substantially from the AboR3 family found in GC1 isolates. Tn6166 can be derived from Tn6167 via a Tn6022-mediated adjacent deletion, leading to loss of the segment between the internal end of Tn6022Δ1 and the end of the tetA gene (compare Figure 1a and b). This derivation suggests that the longer backbone configuration in Tn6167 is ancestral, even though Tn6166 was found in an isolate with an earlier (1982) isolation date.1 Hence, acquisition of the precursor of Tn6167 by an island-free member of the GC2 clone must have occurred early in the course of development of resistance to multiple antibiotics. Changes have also occurred in the O-antigen cluster, indicated by the differences in the gpi gene (allele 7 or 3) and in gyrB (allele 3 or 12).

A possible backbone structure can be deduced by removing ISAba17 and Tn2006, which are likely to be later additions, from Tn6167. A description of the comM transposon in the genome of the South Korean A. baumannii strain 1656-213 has not been published. However, our analysis revealed that it is a close relative of Tn6167 that lacks both ISAba17 and Tn2006. In addition, the segment that includes the tet(B) determinant of Tn10 and the fragment of the small mobile element CR2 has been lost from the 1656-2 structure, presumably via homologous recombination between the two copies of the duplicated portion of CR2. The 1656-2 transposon has acquired additional resistance genes in a 6.9 kb segment containing the blaOER-1 gene associated with a copy of the strA gene that appears to have been incorporated by homologous recombination between the incoming strA region and the strA gene in the backbone transposon.

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Transparency declarations
None to declare.

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