A novel family of genomic resistance islands, AbGRI2, contributing to aminoglycoside resistance in Acinetobacter baumannii isolates belonging to global clone 2

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Objectives: To determine the context and location of antibiotic resistance genes in carbapenem- and aminoglycoside-resistant Acinetobacter baumannii global clone 2 (GC2) isolates carrying a class 1 integron.

Methods: Isolates were from Sydney hospitals. Resistance to antibiotics was determined by disc diffusion. BLAST searches identified relevant DNA fragments in a draft genome sequence. PCR was used to assemble fragments and map equivalent regions.

Results: In two isolates belonging to GC2, WM99c and A91, the \(\text{bla}\) gene, the class 1 integron carrying the \(\text{aacC1-orfP-orfP-orfQ-aadA1}\) cassette array and \(\text{sul1}\) gene, and the \(\text{aphA1b}\) gene in Tn\(6020\) were each in segments flanked by IS\(26\). These, together with a fourth IS\(26\)-flanked segment, formed a 19.5 kb genomic resistance island (GRI), designated AbGRI2-1, containing five copies of IS\(26\). Part of this island was identical to part of the multiple antibiotic resistance region of AbaR-type islands found in global clone 1 (GC1). AbGRI2-1 has replaced a 40.9 kb segment found in the AB0057 genome. Related GRIs were identified in the same location in published GC2 genomes and appear to have arisen from AbGRI2-1 via IS\(26\)-mediated deletions. Like A91, WM99c carries ISAba1 upstream of \(\text{ampC}\) and Tn\(6167\), an AbGRI1-type island in the chromosomal \(\text{comM}\) gene containing \(\text{sul2, tet(B), strA}\) and \(\text{strB}\) genes and \(\text{bla}_{\text{OXA-23}}\) in Tn\(2006\). In WM99c, the chromosomal gene encoding OXA-Ab is interrupted by ISAba17.

Conclusions: AbGRI2-1 is the largest so far of a new type of GRI designated AbGRI2 to distinguish them from the islands in \(\text{comM}\) in GC1 isolates (AbaR type) and in GC2 isolates (AbGRI1 type).

Keywords: gentamicin-resistant A. baumannii, class 1 integrons, Tn6020

Introduction

Two globally disseminated clonal complexes, global clones 1 and 2 (GC1 and GC2), include the majority of Acinetobacter baumannii that are resistant to multiple antibiotics.1–3 However, within each clone resistance has gradually accumulated via multiple routes and there is substantial variation in resistance profiles and resistance gene content. In addition to resistance to cephalosporins and carbapenems, many strains carry aminoglycoside resistance genes.2–4,9 A class 1 integron carrying the \(\text{aacC1-orfP-orfP-orfQ-aadA1}\) gene cassette, conferring gentamicin resistance, in an \(\text{aacC1-orfP-orfQ-aadA1}\) or \(\text{aacC1-orfP-orfQ-aadA1}\) array is very prevalent in GC1 isolates, but is also found in some GC2 isolates. The \(\text{aphA1b}\) gene (neomycin and kanamycin resistance), flanked by two directly oriented copies of IS\(26\) in the transposon Tn\(6020\),10 has also been found together with \(\text{aacC1}\) in both GC1 and GC2 isolates.2–8

In GC1 strains, the integron with \(\text{aacC1-orfP-orfQ-aadA1}\) and Tn\(6020\) are usually present and are adjacent to one another within an AbaR-type resistance island, which is located in the chromosomal \(\text{comM}\) gene.2,11,12 However, studies focusing on aminoglycoside resistance in GC2 isolates collected from multiple hospitals from Australia’s eastern seaboard showed that only a subgroup of GC2 isolates contained aacC1-orfP-orfQ-aadA1 along with Tn\(6020\).2,7,8 In A91, a representative of this group, the resistance island in \(\text{comM}\) does not contain either the class 1 integron or Tn\(6020\).13 Instead, it has a different structure and contains sul2, tet(B), strA and strB (resistance to

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sulphonamides, tetracycline, streptomycin) as well as *bla*$_{OXA-23}$, conerring resistance to carbapenens, in Tn2006. Several other GC2 strains carry related islands in comM that lack Tn2006.**16**–**17**

WM99c is a 1999 *A. baumannii* isolate from Westmead Hospital in Sydney that was shown to be carbapenem resistant due to production of the OXA-23 carbapenemase and gentamicin resistant due to the *aacC1-orfP-orfP-orfQ-orfQ*-aadA1 cassette array.**9** WM99c was recently shown to belong to GC2 and a draft genome sequence assembly was reported (GenBank accession number AERY00000000).**18** Here, we have examined the resistance gene content and context in WM99c and determined the structure of a novel resistance island.

**Materials and methods**

**Isolate characterization**

The reported**9** resistance profile of WM99c, a multiply resistant *A. baumannii* isolate from Westmead Hospital, Sydney, was confirmed and extended as described previously.**8** The sequence type (ST), according to multilocus sequence typing (MLST) schemes hosted at Oxford University (http://pubmlst.org/abauumannii/) and Institut Pasteur (www.pasteur.fr/mlst), was determined from genome sequence data and re-sequencing of the gpi allele.

**Genome sequence analysis**

The island inserted in the chromosomal comM gene was mapped using previously described primers**13,14** to join five contigs identified in draft genome sequence AERY00000000**15** with the sequence of Tn6167 (GenBank accession number JN968483). Contigs 00164, 00027, 00026, 00043, 00025 and 00060 containing the remaining resistance genes (*bla*$_{TEM}$, *aphA1*, *aacC1*, *sul1*) were identified using pairwise alignments with standard sequences. As duplicated sequence had been trimmed from the available contigs, BLAST searches of a database composed of the 454 reads with the sequences of IS26, ISAba1 and ISAba17 were used to identify insertion sequence (IS)-containing sequences. Sequences flanking IS were then identified and matched to the available contigs in the WM99c draft genome using BLAST. These contigs were then assembled using PCR mapping with the primers listed in Table 1 and closed by sequencing the amplicons. PCR conditions used to detect short and long amplicons were described previously.**17** Additional sequences were determined as described previously, and assembled using Sequencer 5.0.1 (Gene Codes Corporation, Ann Arbor, MI, USA). Gene Construction Kit version 2.5 (Textco, West Lebanon, NH, USA) was used to draw the figure to scale.

**GenBank accession number**

A 21477 bp sequence from WM99c that includes the 19.5 kb resistance island containing *bla*$_{TEM}$, *aphA1b*, *aacC1*, *sul1* and *sul2* with some flanking DNA has been submitted to GenBank under accession number JX869489. This sequence overlaps contigs 00164 and 00024 in the draft genome sequence AERY00000000.

**Results and discussion**

**WM99c resembles other Australian GC2 isolates**

From the available draft genome sequence, WM99c was found to be ST2 (2-2-2-2-2-2; Institut Pasteur scheme) and ST92 (1-3-3-2-2-7-3; Oxford University scheme), confirming it was a member of GC2. WM99c was previously shown to be resistant to imipenem, meropenem, ampicillin, cefotaxime, cefazidime, cefotin, aztreonam, ticarcillin, gentamicin and ciprofloxacin, while susceptible to amikacin and tobramycin.**9** WM99c is also resistant to tigecycline.**20** Here, it was also found to be resistant to sulfamethoxazole, streptomycin, spectinomycin, tetracycline, kanamycin, neomycin and nalidixic acid. This phenotype was identical to that observed previously in several OXA-23-producing GC2 isolates containing aacC1-orfP-orfP-orfQ-orfQ-aadA1 cassettes and Tn6020 from other Australian hospitals.**8** Additional resistance genes, *aphA1b*, *bla*$_{TEM}$-*sul1*, *sul2*, *strA* and *strB* and the tet(B) determinant, found in these isolates were identified in the WM99c draft genome sequence. An ISAba1 was found upstream of the chromosomal *ampC* gene, contributing to resistance to third-generation cephalosporins. WM99c had been shown to have a large interruption in the chromosomal *bla*$_{OXA-23}$ gene, and ISAba17, a 2.5 kb IS, was found in this gene.

**Structure of the genomic resistance islands (GRIs)**

Isolate A91, a representative of the Australian GC2 isolates described above, also belongs to ST92 and includes Tn6167 (GenBank Accession number JN968483) in the chromosomal comM gene. Tn6167 contains the *sul2*, *strA* and *strB* genes, the tet(B) determinant and *bla*$_{OXA-23}$ in Tn2006. Five contigs that matched Tn6167 were found in the WM99c draft genome. PCRs described previously**13** ordered and linked these contigs, showing that WM99c contained a resistance island identical to Tn6167 in the comM gene.

The remaining resistance genes were mapped to a second novel GRI. Contigs containing the class 1 integron with the gene cassettes and *sul1* gene, or the *aphA1b* or *bla*$_{TEM}$ genes were identified and these three segments were each flanked by two copies of IS26. The IS26-flanked segments were ordered and assembled using PCR and joined by sequencing the linkage products. The complete 19.5 kb structure, which contains a fourth IS26-flanked

<table>
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<tr>
<th>Table 1. Primers</th>
<th>Primer location</th>
<th>Sequence (5′–3′)</th>
<th>Predicted product size (bp)</th>
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<td>tnpR$_{2}$</td>
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*aLoci in the genome of AB0057 (GenBank accession number CP001182).*
segment and all five copies of IS26 found in the genome, is shown in Figure 1(a). Like the multiple antibiotic resistance region of AbaR,10 this GRI is a mosaic of segments with different origins (indicated in Figure 1a). Indeed, a 9 kb region spanning from within Tn1 to the end of Tn6020 (indicated in Figure 1a) is identical to a part of the AbaR region of several GC1 isolates (see Post et al.,2 Post et al.,10 Krizova et al.11 and Post et al.12). However, though the integron containing the aacC1-orfP-orfP-orfQ-aadA1 array is also adjacent to Tn6020 in the AbaR islands, in the WM99c GRI the integron is in the opposite orientation. The integron-derived region is also larger and is a remnant of an In4-type integron that includes the 5′- and 3′-conserved segments, but only part of IS6100. It is located adjacent to an incomplete Tn21 transposition module (Figure 1a).

PCR mapping using primers shown in Table 1 demonstrated that isolate A91, which is from a different Sydney hospital, contained the same aminoglycoside resistance island. Hence, the two isolates are closely related.

This novel resistance island type was named AbGRI2 to distinguish it from the resistance islands found in comM and related to Tn6167 (here designated the AbGRI1 type). As the first and largest identified member of the AbGRI2 resistance island group, the island identified in WM99c and A91 was named AbGRI2-1.

Location of AbGRI2-1

Two further contigs that abut an IS26 (00164 and 00024 in GenBank accession number AERY00000000) were shown to be located on either side of AbGRI2-1, with 00024 on the right and 00164 on the left [orientation as shown in Figure 1(a)]. AbGRI2-1 replaces a 40.9 kb segment found in the genomes of the GC1 strains AB0057 (1258357–1299260 in GenBank accession number CP001182) and AB307-0294 (2568231–2609132 in GenBank accession number CP0001172). ACICU (GenBank accession number CP000863), the first fully sequenced GC2 isolate, contains a single copy of IS26 in this vicinity that has replaced a 38.2 kb segment (1263138–1301378 in AB0057) that partly overlaps the segment lost in WM99c.

Other AbGRI2 group islands

The genomes of completely sequenced GC2 strains were examined for the presence of IS26 or a GRI in the same location as AbGRI2-1. Strain MDR-TJ (GenBank accession number CP003500)17 has a resistance region, here designated AbGRI2-2, that is in the same position and resembles the one in WM99c and A91 (Figure 1b). MDR-TJ lacks the segments containing the blaTEM and aphA1b genes.
(Figure 1b), suggesting they have been lost in a precise way. MDR-ZJ06 (GenBank accession number CP001937) contains AbGRI2-3 with only the integrase-containing segment at this location, but it is inverted and the cassette array has been replaced with aacA4-catB8-aadA1 (Figure 1c). In addition a 7.8 kb segment of chromosomal DNA adjacent to the IS26 on the left (as shown in Figure 1) has been deleted.

1656-2 (GenBank accession number CP001921) has a truncated version of the integrase-containing segment that includes only intI1-aacC1-orfP-orfQΔ flanked by two copies of IS26 (not shown). However, in 1656-2 the right-hand IS26 is in the same position as in other AbGRI2 variants, but the left-hand IS26 is adjacent to a chromosomal region that is located 1.4 Mb away in the MDR-TJ and MDR-ZJ06 chromosomes. Finally, TCDC-AB0715 (GenBank accession number CP002522) contains an insertion at the same position as AbGRI2-1 with a complete copy of IS26 on the right and a partial copy on the left. However, the intervening sequence consists of fragments of IS26 and non-coding DNA from Tn6020. Whether this represents an assembly error remains to be established.

The MLST types in the Oxford University scheme deduced from the genome sequences described above were different from WM99c and A91 and from one another due to different gpi alleles.

Conclusions
A new AbGRI2 family of islands has been identified here in the genomes of GC2 isolates. The presence of multiple copies of IS26 has led to a series of derivatives that arose either via precise loss or gain of a segment located between two IS26 copies together with one IS26, or via IS26-mediated deletion of adjacent DNA. AbGRI2 appears to have entered the GC2 lineage prior to the genetic changes that altered the gpi allele, leading to different MLST types.

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Transparency declarations
None to declare.

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