

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 *bla*_{TEM} gene, the class 1 integron carrying the *aacC1*-*orfP*-*orfQ*-*aadA1* cassette array and *sul1* gene, and the *aphA1b* gene in Tn6020 were each in segments flanked by IS26. These, together with a fourth IS26-flanked segment, formed a 19.5 kb genomic resistance island (GRI), designated AbGRI2-1, containing five copies of IS26. 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 IS26-mediated deletions. Like A91, WM99c carries ISAba1 upstream of *ampC* and Tn6167, an AbGRI1-type island in the chromosomal *comM* gene containing *sul2*, *tet(B)*, *strA* and *strB* genes and *bla*_{OXA-23} in Tn2006. 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 *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 *aacC1* gene cassette, conferring gentamicin resistance, in an *aacC1*-*orfP*-*orfQ*-*aadA1* (or *aacC1*-*orfP*-*orfQ*-*aadA1*) array is very prevalent in GC1 isolates, but is also found in some GC2 isolates. The *aphA1b* gene (neomycin and kanamycin resistance), flanked

by two directly oriented copies of IS26 in the transposon Tn6020,¹⁰ has also been found together with *aacC1* in both GC1 and GC2 isolates.^{2,8}

In GC1 strains, the integron with *aacC1*-*orfP*-(*orfP*-)*orfQ*-*aadA1* and Tn6020 are usually present and are adjacent to one another within an AbaR-type resistance island, which is located in the chromosomal *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 Tn6020.^{2,7,8} In A91, a representative of this group, the resistance island in *comM* does not contain either the class 1 integron or Tn6020.¹³ Instead, it has a different structure and contains *sul2*, *tet(B)*, *strA* and *strB* (resistance to

sulphonamides, tetracycline, streptomycin) as well as *bla*_{OXA-23}, conferring resistance to carbapenems, in Tn2006. Several other GC2 strains carry related islands in *comM* that lack Tn2006.^{14–17}

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-*aadA1* cassette array.⁹ WM99c was recently shown to belong to GC2 and a draft genome sequence assembly was reported (GenBank accession number AERY000000000).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 reported⁹ 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/abaumannii/>) 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 AERY000000000¹⁸ 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, ISAb1 and ISAb17 were used to identify insertion sequence (IS)-containing sequences. Sequences

Table 1. Primers

| PCR | Primer | Primer location | Sequence (5'–3') | Predicted product size (bp) |
|-----|--------|------------------------------|------------------------|-----------------------------|
| 1 | RH1315 | AB57_1175 ^a | AGGAGATCTTCTGGCAGTCA | 1051 |
| | RH539 | <i>tnpR</i> ₁ | CCAGCCCTTCCCAGATCTGTTG | |
| 2 | RH605 | <i>bla</i> _{TEM} | TTTCGTGTCGCCCTTATTCC | 2650 |
| | RH759 | <i>tnpA</i> ₁₀₀₀ | GCCAGCTCATTACCTTGCCGA | |
| 3 | RH1315 | AB57_1175 ^a | AGGAGATCTTCTGGCAGTCA | 4308 |
| | RH759 | <i>tnpA</i> ₁₀₀₀ | GCCAGCTCATTACCTTGCCGA | |
| 4 | RH520 | <i>tnpR</i> _{5393c} | CATGGCCCAGCGGATACTTCAG | 2297 |
| | RH880 | <i>aphA1</i> | CAACGGGAAACGTCTTGCTC | |
| 5 | RH881 | <i>aphA1</i> | ATTCGTGATTGCGCCTGAG | 2712 |
| | RH751 | <i>sul1</i> | GCGGAACCTCACGCGATC | |
| 6 | RH520 | <i>tnpR</i> _{5393c} | CATGGCCCAGCGGATACTTCAG | 4554 |
| | RH751 | <i>sul1</i> | GCGGAACCTCACGCGATC | |
| 7 | RH668 | <i>tnpA</i> ₂₁ | CACCAGAACCGCCTGCTCAA | 1219 |
| | RH1316 | AB57_1209 ^a | CATCTGCCATCCAGTTTGTG | |

^aLoci in the genome of AB0057 (GenBank accession number CP001182).

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.¹⁹ Additional sequences were determined as described previously, and assembled using Sequencher 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* in Tn6020, *aacC1*-orfP-orfP-orfQ-*aadA1* and *sul1* 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 AERY000000000.

Results and discussion

WM99c resembles other Australian GC2 isolates

From the available draft genome sequence, WM99c was found to be ST2 (2-2-2-2-2-2-2; 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, ceftazidime, cefalotin, 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-*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 ISAb1 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-Ab} gene,⁹ and ISAb17, a 2.5 kb IS,¹³ 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 previously¹³ 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

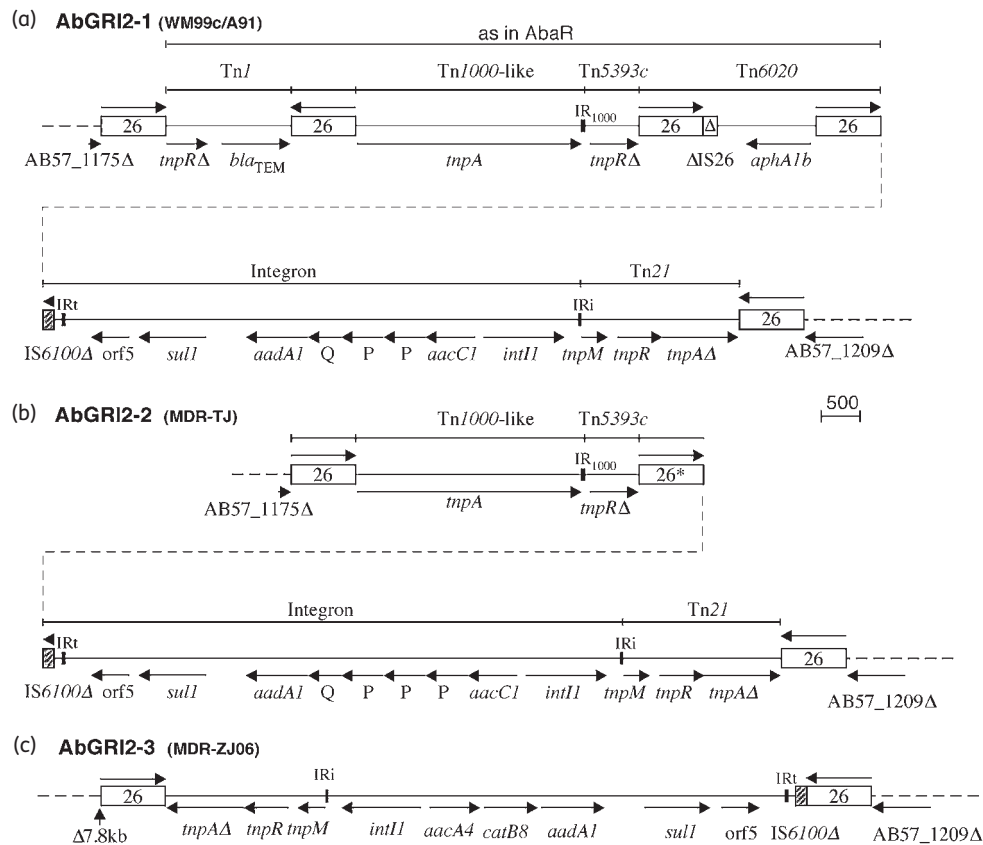


Figure 1. Structures of AbGRI2 variants. (a) AbGRI2-1 from WM99c and A91. (b) AbGRI2-1 from MDR-TJ. (c) AbGRI2-3 from MDR-ZJ06. The resistance island is indicated by a solid line with copies of IS26 shown as open boxes with arrows above indicating the orientation of the *tnp* transposase gene, and inverted repeats are shown as vertical lines. A grey box represents a fragment of IS6100. The extent and orientation of genes and open reading frames is indicated by arrows below. Chromosomal DNA is depicted by dashed lines with the AB0057 locus tags of the interrupted genes indicated below. In (a) and (b), the origin of different DNA segments is indicated above, and in (a) the segment found in AbaR-type islands is also indicated above.

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*-*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 integron²¹ 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 (1 258 357–1 299 260 in GenBank accession number CP001182) and AB307-0294 (2 568 231–2 609 132 in GenBank accession number CP001172). 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 (1 263 138–1 301 378 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 *bla_{TEM}* 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 integron-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 integron-containing segment that includes only *intI1Δ-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.

References

- Diancourt L, Passet V, Nemeč A *et al.* The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS One* 2010; **5**: e10034.
- Post V, White PA, Hall RM. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; **16**: 1162–70.
- Mugnier PD, Poirel L, Naas T *et al.* Worldwide dissemination of the *bla*_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 2010; **16**: 35–40.
- Seward RJ, Lambert T, Towner KJ. Molecular epidemiology of aminoglycoside resistance in *Acinetobacter* spp. *J Med Microbiol* 1998; **47**: 455–62.
- Gombac F, Riccio ML, Rossolini GM *et al.* Molecular characterization of integrons in epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* from Italian hospitals reveals a limited diversity of gene cassette arrays. *Antimicrob Agents Chemother* 2002; **46**: 3665–8.
- Nemeč A, Dolzani L, Brisse S *et al.* Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of pan-European *Acinetobacter baumannii* clones. *J Med Microbiol* 2004; **53**: 1233–40.
- Mak JK, Kim MJ, Pham J *et al.* Antibiotic resistance determinants in nosocomial strains of multidrug-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2009; **63**: 47–54.
- Nigro SJ, Post V, Hall RM. Aminoglycoside resistance in multiply antibiotic-resistant *Acinetobacter baumannii* belonging to global clone 2 from Australian hospitals. *J Antimicrob Chemother* 2011; **66**: 1504–9.
- Valenzuela JK, Thomas L, Partridge SR *et al.* Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. *J Clin Microbiol* 2007; **45**: 453–60.
- Post V, Hall RM. AbaR5, a large multiple antibiotic resistance region found in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; **53**: 2667–71.
- Krizova L, Dijkshoorn L, Nemeč A. Diversity and evolution of AbaR genomic resistance islands in *Acinetobacter baumannii* strains of European clone I. *Antimicrob Agents Chemother* 2011; **55**: 3201–6.
- Post V, Hamidian M, Hall RM. Antibiotic-resistant *Acinetobacter baumannii* variants belonging to global clone 1. *J Antimicrob Chemother* 2012; **67**: 1039–40.
- Nigro SJ, Hall RM. Tn6167, an antibiotic resistance island in an Australian carbapenem-resistant *Acinetobacter baumannii* GC2, ST92 isolate. *J Antimicrob Chemother* 2012; **67**: 1342–6.
- Nigro SJ, Hall RM. Antibiotic resistance islands in A320 (RUH134), the reference strain for *Acinetobacter baumannii* global clone 2. *J Antimicrob Chemother* 2012; **67**: 335–8.
- Seputiene V, Povilonis J, Suziedeliene E. Novel variants of AbaR resistance islands with a common backbone in *Acinetobacter baumannii* isolates of European clone II. *Antimicrob Agents Chemother* 2012; **56**: 1969–73.
- Zhou H, Zhang T, Yu D *et al.* Genomic analysis of the multidrug-resistant *Acinetobacter baumannii* strain MDR-ZJ06 widely spread in China. *Antimicrob Agents Chemother* 2011; **55**: 4506–12.
- Huang H, Yang Z-L, Wu X-M *et al.* Complete genome sequence of *Acinetobacter baumannii* MDR-TJ and insights into its mechanism of antibiotic resistance. *J Antimicrob Chemother* 2012; **67**: 2825–32.
- Eijkelkamp BA, Stroehrer UH, Hassan KA *et al.* Adherence and motility characteristics of clinical *Acinetobacter baumannii* isolates. *FEMS Microbiol Lett* 2011; **323**: 44–51.
- Hamidian M, Hall RM. AbaR4 replaces AbaR3 in a carbapenem-resistant *Acinetobacter baumannii* isolate belonging to global clone 1 from an Australian hospital. *J Antimicrob Chemother* 2011; **66**: 2484–91.
- Iredell J, Thomas L, Power D *et al.* Tigecycline resistance in Australian antibiotic-resistant Gram-negative bacteria. *J Antimicrob Chemother* 2007; **59**: 816–8.
- Partridge SR, Recchia GD, Stokes HW *et al.* Family of class 1 integrons related to In4 from Tn1696. *Antimicrob Agents Chemother* 2001; **45**: 3014–20.