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Amikacin resistance plasmids in extensively antibiotic-resistant GC2 *Acinetobacter baumannii* from two Australian hospitals

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Sir,

Previously, we studied a collection of extensively antibiotic-resistant *Acinetobacter baumannii* belonging to global clone 2 (GC2) from Australian hospitals in Sydney, Brisbane and Canberra.[1] These 61 closely related carbapenem-resistant isolates shared a common set of resistance genes, but were subdivided into five different groups based on their aminoglycoside resistance phenotypes. One group harboured *aacC1* (gentamicin resistance), *aphA1* in Tn*6020* (kanamycin and neomycin resistance) and *aphA6* in Tn*aphA6* (amikacin, kanamycin and neomycin resistance). We subsequently showed that *aacC1* and *aphA1* were located in a chromosomal resistance island, AbGRI2-1, in two representatives of this collection that did not carry Tn*aphA6*.[2]

The *aphA6* gene was originally recovered from an *A. baumannii* isolate on a 63 kb plasmid that was transferrable to other *Acinetobacter* spp.[3] However, this plasmid was not sequenced. Recently, Tn*aphA6* was found on a completely sequenced 70 kb conjugative plasmid, pAb-G7-2 (GenBank accession number KF669606),[4] which was in a GC1 isolate from Melbourne. pAb-G7-2 belongs to the *rep*Aci6 plasmid family.[4] The archetype of the *rep*Aci6 family,[5] pACICU2, was originally reported to be cryptic.[6] However, it was recently shown to carry Tn*aphA6* in the same position as pAb-G7-2 and to transfer amikacin resistance.[7]

Here, we have examined whether the Tn*aphA6* in the GC2 isolates, D72 and C20, which also harbour *aacC1* and *aphA1*, may also be on a conjugative *rep*Aci6 family plasmid. While D72 and C20 had the same resistance phenotype and gene content,[1] they were isolated from different Sydney hospitals 8 years apart, C20 in 2002 and D72 in 2010.

To determine whether C20 and D72 harboured pAb-G7-2, the PCR mapping strategy described previously was used.[4] For C20, all of the PCRs produced amplicons identical to those in pAb-G7-2, indicating that C20 contained a plasmid with Tn*aphA6* in the same position. A draft genome sequence for C20 was subsequently generated at the Wellcome Trust Sanger Institute using Illumina HiSeq.[8] A standalone BLAST[9] search using pAb-G7-2 as a query retrieved a 68 kb contig that matched pAb-G7-2 and a contig containing most of Tn*aphA6*. The assembled *rep*Aci6 plasmid in C20 was identical to pAb-G7-2.

In D72, the plasmid backbone was present, but was not interrupted at the Tn*aphA6* position in pAb-G7-2, and this was confirmed using the draft genome sequence of D72 (Figure 1). However, two plasmid backbone contigs ended with a fragment of ISAba125, which flanks *aphA6* in Tn*aphA6*. Primers adjacent to the ISAba125 pieces, RH1319 (TTCCATTGCATCGTTTGAGA) and RH1320 (TTTACGATAATTGCATGGATGT), amplified a 3.3 kb product, indicating that Tn*aphA6* was at a different position relative to that in pAb-G7-2 (Figure 1). PCRs linking RH1319 and RH1320 to *aphA6* produced amplicons of the size predicted if Tn*aphA6* was present at this second location. The complete sequence of a 70102 bp plasmid in D72 was assembled as described previously[4] for pAb-G7-2 (Figure 1) and was deposited into GenBank under the accession number KM051846. This plasmid was named pD72-2.

The backbones of pD72-2 and pAb-G7-2 shared 99.9% sequence identity. There were 61 nucleotide differences, four single-base gaps and one two-base gap in pD72-2. There was a single nucleotide difference between the *rep*Aci6 genes that resulted in a single amino acid change (L344I) and a single additional base in the third of the three previously defined repeat regions[4] (3 in Figure 1). Comparison of pD72-2 with pA85,[10] a closely related *rep*Aci6 plasmid that does not contain Tn*aphA6*, but harbours bla_{OXA-23} in AbaR4, showed that the pD72-2 backbone was smaller, suggesting that there are a number of separate sublineages in the *rep*Aci6 plasmid family.

Both D72 and C20 also included a small 10967 bp cryptic *rep*Aci2 plasmid that carries a mobilization gene and a single ISAba125. This plasmid was named pD72-1 (GenBank accession number KM051986). C20 harboured a second 110967 bp cryptic plasmid that shared 99.99% identity (six single-base changes) with pABTJ2 (GenBank accession number CP004359). The *rep* gene of pABTJ2 did not fit within one of the previously defined *A. baumannii rep* families,[5] and its Rep protein of 373 amino acids shared only 41% amino acid identity with a 218 amino acid segment of RepAci2 (316 amino acids).

Assembly of contigs in the draft genomes of C20 and D72 using PCR showed that they both contained AbGRI2. C20 harboured an island identical to AbGRI2-1, which has *aacC1* and *aphA1*, while D72 had a variant of AbGRI2-1 with at least one additional copy of *aphA1*. Both C20 and D72 also had a chromosomal resistance island in *comM* that was identical to Tn*6167*,[11] here renamed AbGRI1-2. The bla_{OXA-23} gene, conferring resistance to carbapenems, is within Tn*2006* in AbGRI1-2.

This study shows that Tn*aphA6* has been acquired by almost identical *rep*Aci6 plasmids on at least two separate occasions. Screening for the position of Tn*aphA6* in these plasmids should be a valuable epidemiological tool and aid in tracking the spread of these conjugative resistance plasmids.

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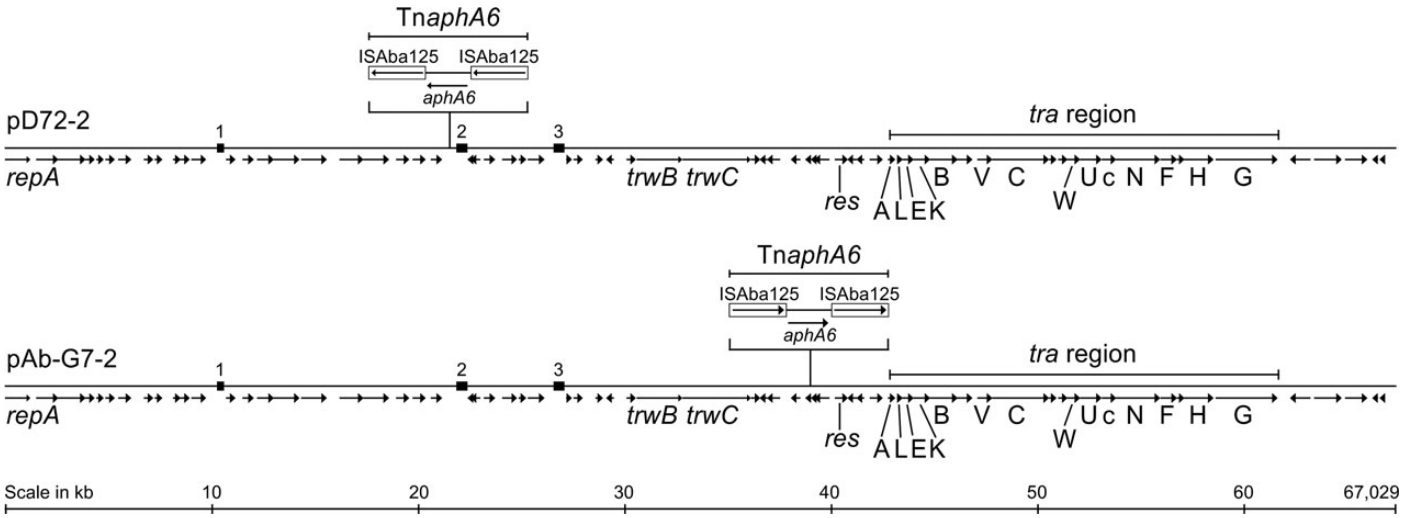

Figure 1. Comparison of pD72-2 and pAb-G7-2. The horizontal lines represent the plasmid backbone opened at the beginning of *repA*. The extents of genes and ORFs are indicated below with names of identifiable genes indicated. The extent of the transfer region is shown above the backbone, respective *tra* genes are indicated with capital letters and *trbC* is represented by c. The three repeat regions are shown as numbered boxes. Tn*aphA6* is shown above the backbone with ISAba125 represented as an open box containing an arrow that shows its orientation. A scale bar for the backbone is shown below the plasmids. Drawn to scale from GenBank accession numbers KM051846 and KF669606.

Transparency declarations

None to declare.

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