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Original Research Article

The extensively antibiotic resistant ST111 *Acinetobacter baumannii* isolate RBH2 carries an extensive mobile element complement of plasmids, transposons and insertion sequences

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ABSTRACT

The complete genome of RBH2, a sporadic, carbapenem resistant ST111 *Acinetobacter baumannii* isolate from Brisbane, Australia was determined and analysed. RBH2 is extensively resistant and the chromosome includes two transposons carrying antibiotic resistance genes, AbaR4 (*oxa23* in Tn*2006*) and Tn*7*::Tn*2006* (*dfrA1*, *sat2*, *aadA1*, *oxa23*). The chromosome also includes two copies of Tn*6175,* a transposon carrying putative copper resistance genes, and 1–17 copies of six different insertion sequences. RBH2 has six plasmids ranging in size from 6 kb – 141 kb, four carrying antibiotic resistance genes. Plasmids pRBH2–1 (*aadB*) and pRBH2–2 (*aphA6* in Tn*aphA6*) were found to be essentially identical to known plasmids pRAY*-v1 and pS21–1, respectively. The largest plasmids, pRBH2–5 (*oxa23* in AbaR4) and pRBH2–6 (*oxa23* in AbaR4::ISAba11 and *sul2*, *tet*(B), *strA and strB* in Tn*6172*) have known transfer-proficient relatives. pRBH2–5, an RP-T1 (RepAci6) plasmid, also carries a different putative copper resistance transposon related to Tn*6177* found in pS21–2. The backbone of pRBH2–5 is related to those of previously described RepAci6 plasmids pAb-G7–2 and pA85–3 but has some distinctive features. Three different RepAci6 backbone types were distinguished, Type 1 (pAb-G7–2), Type 2 (pA85–3) and Type 3 (pRBH2–5 and pS21–2). pRBH2–6 is closely related to pAB3 and their backbones differ by only 5 SNPs. Plasmids pRBH2–3 and pRBH2–4 do not carry antibiotic resistance genes. pRBH2–3 does not include an identifiable *rep* gene and is a novel plasmid type. pRBH2–4 is of the R3-T3 type and includes segments of the larger pABTJ2 that heads this group. Other ST111 genomes carry different plasmids.

1. Introduction

Whereas in most Gram-negative bacterial pathogens the antibiotic resistance genes are generally found in plasmids, in the dominant globally disseminated clonal complexes of *Acinetobacter baumannii*, namely GC1 and GC2, the same resistance genes are mainly found in specific acquired regions in the chromosome (Adams et al., 2008; Post et al., 2010; Blackwell et al., 2016; Holt et al., 2016; Nigro and Hall, 2016; Hamidian and Hall, 2018b; Chan et al., 2020; Hua et al., 2021). This is partly explained by the fact that the plasmids that carry the resistance genes in *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* and other enteric pathogens are not found in *Acinetobacter* species; presumably they are unable to survive because they are unable to replicate. Likewise, plasmids found in *Acinetobacter* are not seen in other Gram-negative species. Nonetheless, plasmids do contribute to the carriage of the resistance gene complement of GC1 and GC2 isolates. The most important of these are pRAY* (Hamidian et al., 2012), conjugative RepAci6 plasmids (Hamidian et al., 2014b; Hamidian et al., 2014a), large conjugative plasmids related to either pAB3 (Weber et al., 2015) and pA297–3 (Hamidian et al., 2016), or to the NDM plasmids (Hu et al., 2012).

A very large number of types of plasmids associated with *A. baumannii* have been identified and many carry *rep* genes encoding replication initiation proteins of the Rep_1, Rep_3 and Rep Pfam groups (Bertini et al., 2010; Lam et al., 2023). Others including some large conjugative plasmids do not contain an identifiable *rep* gene (Hu et al., 2012; Weber et al., 2015; Hamidian et al., 2016; Lam et al., 2023). A number of schemes have been proposed for the classification of these plasmids (Bertini et al., 2010; Mindlin et al., 2020; Salgado-Camargo et al., 2020), the most recent typed the known plasmids with a *rep* gene

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into a total of 80 types (Lam et al., 2023).

Less attention has been paid to how non-GC1, non-GC2 *A. baumannii* isolates have become extensively antibiotic resistant. RBH2 is an early extensively resistant isolate from Brisbane recovered in 1999 and belongs to a rare sequence type, ST111 in the Institut Pastuer scheme and ST1134 in the Oxford MLST scheme (Kenyon et al., 2016; Hamidian et al., 2017). ST111 shares no more than three of the seven MLST alleles with the more prevalent GC1, GC2 and ST25 groups. The capsule locus of RBH2 has been previously characterised as KL19 with the *wzy* gene for the K unit polymerase found in an additional small genomic island (6094 bp) at the 3′-end of *cpn60* that also includes the acetyltransferase gene *atr25*, interrupted by an ISAba1 (Kenyon et al., 2016; Genbank Accession KU165787).

In this study, the complete genome of the RBH2 isolate was determined and the plasmids and transposons were examined. The context of resistance genes in the chromosome and plasmids was also examined and compared to similar regions in sequences available in the GenBank nucleotide database.

2. Materials and methods

2.1. Bacterial isolate and antibiotic susceptibility testing

A. baumannii strain RBH2 is an extensively antibiotic resistant isolate recovered from a patient at the Royal Brisbane and Women's Hospital in Queensland, Australia in 1999. RBH2 was kindly supplied by Dr. Mohammad Katouli and is derived from the large collection described by Runnegar et al. (Runnegar et al., 2010). RBH2 was screened for resistance to 28 antibiotics using a calibrated disc diffusion assay. Antibiotic discs (Oxoid) used were ampicillin (25 μg), ampicillin/sulbactam (20 μg), piperacillin/tazobactam (110 μg), imipenem (10 μg), meropenem (10 μg), doripenem (10 μg), cefepime (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), amikacin (30 μg), gentamicin (10 μg), kanamycin (30 μg), neomycin (30 μg), netilmicin (30 μg), streptomycin (25 μg), spectinomycin (25 μg), tobramycin (10 μg), sulfamethoxazole (300 μg), trimethoprim (5 μg), tetracycline (30 μg), minocycline (30 μg), chloramphenicol (30 μg), florfenicol (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg) and rifampicin (30 μg). The minimum inhibitory concentrations (MIC) for colistin and meropenem were determined by Etest (bioMérieux, Durham, NC, USA).

2.2. Genome sequencing

Whole-cell DNA was prepared, quality controlled for length using gel electrophoresis, and quantified with the Qubit (Invitrogen) system as described previously (Nigro et al., 2018). Library preparation, barcoding and MinION (Oxford Nanopore Technologies) sequencing of prepared DNA were performed as described in detail elsewhere (Wick et al., 2017a). Base calling of reads was performed using Albacore (v1.2.5) and reads were trimmed and demultiplexed with Porechop (v0.2.1) as described in Wick et al. (2017a). A total of 12,421 reads were obtained (average length, 9506 bp; 29.6× coverage). Read quality was validated using FastQC (https://qubeshub.org/resources/fastqc).

The MinION reads (SRA accession number SRR22070826) were subsampled for length and quality using Filtlong (v0.1.0) (https://github.com/rrwick/Filtlong) and combined with available 100 bp paired-end Illumina HiSeq reads (57-fold coverage; SRA accession number SRR22070827) using Unicycler Software (V0.4.0) (Wick et al., 2017b) with default parameters to generate a hybrid assembly of the RBH2 genome.

2.3. Bioinformatic analysis and annotation

Plasmids with a *rep* gene were typed using the recently reported typing tool (Lam et al., 2023). Insertion sequences (IS) were identified using ISFinder (https://isfinder.biotoul.fr/). Resistance genes present in RBH2 and the *oxaAb* allele were determined using ResFinder (https://cge.food.dtu.dk/services/ResFinder/). The *ampC* allele was determined using the AmpC allele database (Karah et al., 2017). The outer core of lipooligosaccharide locus (OCL) was identified using Kaptive (Wyres et al., 2020). p*dif* sites and *dif* modules were identified and annotated as described previously (Blackwell and Hall, 2019). The protein coding regions, rRNA and tRNA genes in the assembled RBH2 genome were initially automatically annotated using Prokka (version 1.13.2). Annotations of regions of interest including IS, polysaccharide biosynthesis loci, plasmids, regions containing antibiotic resistance genes, *dif* modules and transposons were enhanced manually. BLAST searches of the GenBank nucleotide database or in-house collections of known resistance gene associated regions using short DNA segments as queries was used to examine the context of antibiotic resistance genes in detail. The average nucleotide identity between RBH2, A1 and WM99c was calculated using the online ANI calculator (https://www.ezbiocloud.net/tools/ani).

2.4. GenBank accession numbers

The sequence data for RBH2 have been deposited in GenBank under BioProject number PRJNA893697 and BioSample number SAMN31494247. Accession numbers for the complete genome of RBH2 are CP110462 for the chromosome, CP110463 for pRBH2–1, CP110464 for pRBH2–2, CP110465 for pRBH2–3, CP110466 for pRBH2–4, CP110467 for pRBH2–5 and CP110468 for pRBH2–6 (See Table 1).

3. Results

3.1. Resistance profile of RBH2

RBH2 has previously been recorded as resistant to gentamicin, tobramycin, amikacin, ciprofloxacin and meropenem (Kenyon et al., 2016). Here, the antibiotic resistance phenotypes were examined using an expanded set of antibiotics (Section 2.1) and RBH2 was found to be resistant to ampicillin, piperacillin/tazobactam, ampicillin/sulbactam, cefotaxime, ceftazidime, ceftriaxone, cefepime, doripenem, imipenem, meropenem, amikacin, gentamicin, kanamycin, neomycin, netilmicin, tobramycin, streptomycin, spectinomycin, sulphamethoxazole, doxycycline, tetracycline, trimethoprim, nalidixic acid, ciprofloxacin and levofloxacin. RBH2 is susceptible to colistin (MIC 0.125 mg/L). The MIC for meropenem was >32 mg/L.

3.2. Complete genome of RBH2

The complete genome of RBH2 assembled from MinION long read sequence data and Illumina HiSeq short-read data consists of the chromosome and 6 plasmids ranging in size from 6 to 141 kb (Table 1). Rep

Table 1
Properties of the RBH2 chromosome and plasmids.

	Size (bp)	Rep[a]	Rep type[b]	Copy number[c]	Accession number
Chromosome	3,983,259	–	–	–	CP110462
pRBH2-1	6078	NR	–	10.43	CP110463
pRBH2–2	12,952	Rep_3	R3-T2	4.58	CP110464
pRBH2–3	75,126	NR	–	1.34	CP110465
pRBH2-4	80,243	Rep_3	R3-T3	1.15	CP110466
pRBH2–5	124,107	RepPriCT_1	RP-T1	1.43	CP110467
pRBH2-6	141,033	NR	–	0.95	CP110468

[a] NR = no known *rep* gene; Rep_3 family (Pfam01051), RepPriCT_1 encode a replicase of the Rep group (Pfam03090) that also include a PriCT_1 motif (Pfam08708).
[b] Rep type according to *rep* typing tool from (Lam et al., 2023).
[c] The copy number was determined from the read depth for the plasmid relative to the read depth of the seven MLST[IP] alleles.

types are also listed in Table 1. Four of the six plasmids in RBH2, pRBH2–1, pRBH2–2, pRBH2–5 and pRBH2–6, were found to carry at least one antibiotic resistance gene (Table 2). The OCL was identified as OCL1, making RBH2 an ST111:ST1134:KL19:OCL1 type isolate. The intrinsic *oxaAb* gene in the chromosome encodes the OXA-88 variant. Acquired resistance genes or alterations affecting chromosomal genes that accounted for all of the observed antibiotic resistance phenotypes were identified and are described in following sections.

3.3. Insertions in the chromosome

Four transposons were found in the chromosome of RBH2 (Fig. 1). Two are identical copies of Tn*6175*, a 37.8 kb putative copper resistance transposon (Hamidian et al., 2021) that is a member of a family of transposons with *tniC*, *tniA*, *tniB* and *tniD* transposition genes that target the *comM* gene (Hamidian and Hall, 2011), and one copy of Tn*6175* is in *comM*. The second copy interrupts a gene annotated as *cobS* which encodes an adenosylcobinamide-GDP ribazoletransferase. The insertion of Tn*6175* has generated a 5 bp target site duplication (TSD) at each location. In RBH2, Tn*6175* has just three single nucleotide differences compared to the copy of Tn*6175* that interrupts *comM* in J9 (Hamidian et al., 2021). The structure of this transposon is discussed in further detail in Section 3.10.

Two transposons carry antibiotic resistance genes. A copy of AbaR4, which includes the *oxa23* carbapenem resistance gene in Tn*2006* (Hamidian and Hall, 2011) and can also target the *comM* gene, was found at a secondary site in the chromosome interrupting an open reading frame that encodes a hypothetical protein (see Fig. 1). AbaR4 is flanked by a 5 bp TSD. A copy of Tn*7* which carries the *aadA1* (streptomycin and spectinomycin resistance), *dfrA1* (trimethoprim resistance) and *sat2* (streptothricin resistance) genes was found at the preferred location downstream of *glmS* in the chromosome (Fig. 1) flanked by a 5 bp TSD. However, another copy of Tn*2006*, carrying *oxa23*, has inserted into the orfX gene cassette found downstream of the *aadA1* gene cassette in Tn*7* generating a 9 bp TSD.

A total of 51 insertion sequences (IS) were found in the chromosome of RBH2, and their locations are shown in Fig. 1. There are 17 copies of ISAba1, including four as part of the copies of Tn*2006* in AbaR4 and in Tn*7*, one upstream of the *ampC* gene that confers resistance to third generation cephalosporins and was identified previously (Hamidian and Hall, 2013), and a copy interrupting *atr25* as noted previously (Kenyon et al., 2016). There were also three copies of ISAba125 and a single copy of IS*26* as well as copies of ISAba9 ($n = 16$), ISAba34 ($n = 3$) and an ISAba12 variant ($n = 11$) (Table 3) which shares 1011/1039 bp (97.31%) DNA identity with the ISAba12 in the ISfinder database across the full IS length, while the transposase shares 298/310 (96.13%) aa identity to the ISAba12 transposase. For simplicity this ISAba12 variant is hereafter named ISAba12-v1.

Table 2
Resistance genes and insertions in plasmids found in RBH2.

Replicon	Resistance genes	Additional transposons	Related plasmids[a]
Chromosome	*dfrA1, aadA1* and *oxa23* in Tn*7*:: Tn*2006*; *oxa23* in AbaR4	Tn*6175* (2)	–
pRBH2–1	*aadB*	–	Identical to pRAY*-v1 from C2 (JF343536)
pRBH2–2	*aphA6* in Tn*aphA6*	–	Identical to pS21–1 (MG954376)
pRBH2–3	–	–	Novel
pRBH2–4	–	–	Part of backbone of pABTJ2 (CP004359)
pRBH2–5	*oxa23* in AbaR4	Tn*6177*-v1	Backbone has 1 SNP compared to pS21–2 (MG954377)
pRBH2–6	*oxa23* in AbaR4:: ISAba11; *sul2*, *tet* (B), *strA and strB* in Tn*6172*	–	Backbone has 3 SNPs and 2 additional bases compared to pAB3 backbone (CP012005)

[a] Sequences of related plasmids were reported in (Hamidian et al., 2012) for pRAY*-v1, (Blackwell and Hall, 2019) for pS21–1 and pS21–2, (Huang et al., 2014) for pABTJ2 and (Weber et al., 2015) for pAB3.

3.4. Antibiotic resistance involving IS induced expression of intrinsic genes

The ISAba1 located upstream of the intrinsic *ampC* gene (allele 41; Karah et al., 2017) leads to the observed cephalosporin resistance of RBH2 (Hamidian and Hall, 2013). Resistance to nalidixic acid is explained by a known mutation in the chromosomal *gyrA* gene, which causes an amino acid substitution in GyrA (S81L). However, a mutation causing a change in the quinolone resistance determining region of ParC (e.g. S84L) leading to ciprofloxacin resistance is not present in RBH2. Hence, other options were sought. An ISAba1 in the *mar* operon was recently identified as the cause of ciprofloxacin resistance in an *A. baumannii* isolate (Harmer et al., 2022) but is not present in RBH2. The presence of an IS upstream of *adeI*, which is part of the AdeIJK efflux system has been shown to increase the MIC for ciprofloxacin (Zlamal et al., 2021) and in RBH2, an ISAba9 is located 24 bp upstream of *adeI*. This likely disrupts the promoter and AdeN repressor binding. In addition, an outward-facing promoter was found in ISAba9 that would support constitutive expression of AdeIJK. In addition, a single base deletion in *adeN* (base 582), the regulator of the AdeIJK efflux system, results in a premature stop codon in the encoded protein causing loss of the last 7 (C-terminal) residues. Loss of these residues is thought to disrupt dimerization and has been shown increase expression of AdeIJK (Rosenfeld et al., 2012). One or both of these changes likely explain the observed ciprofloxacin resistance.

3.5. Plasmids pRBH2–1 and pRBH2–2 carry antibiotic resistance genes

The smallest plasmid, pRBH2–1 (6078 bp) is identical to pRAY*-v1 (JF343536) found previously in another Australian *A. baumannii* isolate C2 recovered at a Sydney Hospital in 2007 (Hamidian et al., 2012). It is a variant of pRAY* which carries the *aadB* resistance gene cassette conferring resistance to gentamicin, tobramycin and kanamycin (Hamidian et al., 2012).

The second small plasmid, pRBH2–2, is identical to the RepAci2 plasmid pS21–1 (MG954376; Blackwell and Hall, 2019), which encodes an R3-T2 type Rep and includes 3 p*dif* sites and 2 *dif* modules. The structure of pS21–1 is described in detail elsewhere (Blackwell and Hall, 2019). pRBH2–2 and pS21–1 carry the *aphA6* aminoglycoside resistance gene that confers resistance to amikacin, kanamycin and neomycin as part of Tn*aphA6* (Nigro and Hall, 2014). Tn*aphA6* is in the same location as ISAba125 in the RepAci2 plasmid pD72–1 (KM051986; Blackwell and Hall, 2019) and pD72–1 may have arisen via loss of the central segment and one IS copy which could occur via homologous recombination as the ISAba125s are directly oriented in Tn*aphA6*.

3.6. pRBH2–3 lacks an identifiable rep gene

pRBH2–3 is a 75,126 bp cryptic plasmid, with 79 open reading frames but no resistance genes (Table 2) or IS (Table 3). The structure is shown in Fig. 2A. No replication gene was identified in the sequence using the *Acinetobacter* plasmid typing database and extensive searches using the product of each reading frame found in the plasmid did not identify any gene that encodes a known type of replication initiation protein. Most reading frames in the plasmid encode hypothetical proteins with no known function (Supplementary Table 1). Two reading frames are annotated as *parA* and *parB* which encode putative plasmid partitioning proteins likely involved in plasmid stability. Other genes are annotated as encoding a DNA polymerase, DNA dependent RNA polymerase, a resolvase, ribonucleotide reductase and Slt

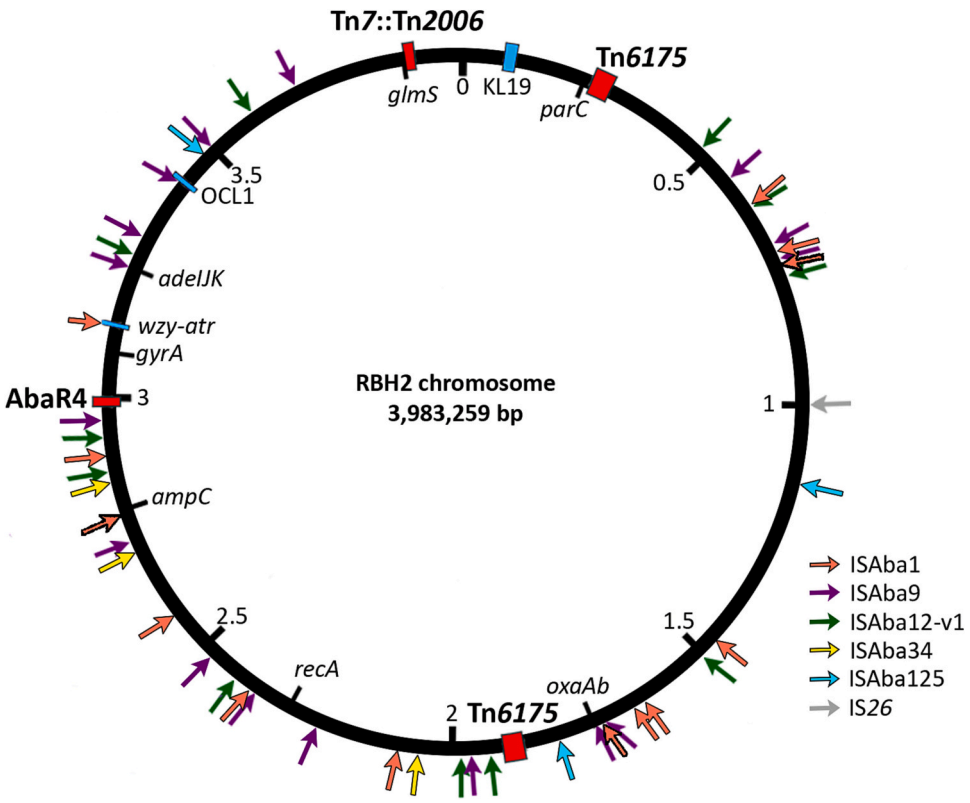


Fig. 1. Location of transposons and insertion sequences in the RBH2 chromosome. Numbers represent the chromosome position in megabases, with 0 indicating the origin of replication. Red boxes indicate the position of transposons Tn*6175*, AbaR4, and Tn*7*::Tn*2006*. Blue boxes indicate the capsule locus (KL19), the *wzy-atr* insertion and the outer core locus (OCL1). The position of some chromosomal genes is shown. Insertion sequences are indicated by arrows and coloured according to the key. The Figure is drawn to scale from GenBank accession number CP110462. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Insertion sequences in RBH2.

IS	IS Family[a]	Chromosome	pRBH2–1	pRBH2–2	pRBH2–3	pRBH2–4	pRBH2–5	pRBH2–6
ISAba1	IS*4*	17	–	–	–	–	3	3
ISAba9	IS*982*	16	–	–	–	–	–	–
ISAba11	IS*701*	–	–	–	–	1	–	1
ISAba12-v1	IS*5*	11	–	–	–	–	1	1 (+ 1Δ)
ISAba34	IS*3*	3	–	–	–	1	–	2
ISAba125	IS*30*	3	–	2	–	–	1	–
IS26	IS*26*	1	–	–	–	1	–	–
IS1006/IS1008	IS*26*	–	–	–	–	1	–	–
IS1006-like	IS*26*	–	–	–	–	1Δ	–	–
IS1008	IS*26*	–	–	–	–	1	–	–
IS17	IS*5*	–	–	–	–	1Δ	–	–
ISAha2	IS*5*	–	–	–	–	1Δ	–	–
Total		**51**	**–**	**2**	**–**	**5 (+3Δ)**	**5**	**7 (+1Δ)**

[a] IS*26* family listed under IS*6* in ISFinder.

transglycosylase. In addition, some genes are annotated as encoding proteins found in phage, for example a N4-g56 family major capsid protein. No conjugative transfer genes were identified.

No related plasmids were identified in the GenBank non-redundant nucleotide database (last searched May 2023). However, searches in the *Acinetobacter* WGS database (last searched May 2023) identified contigs from the draft genomes of 11 *A. baumannii* strains with >95% query coverage and high identity (>97.30%) to pRBH2–3 (Fig. 2B/ Table 4). None of these strains have the same ST^{IP} as RBH2. For all strains, the matching sequence was in a single contig, except for NIPH 1362 where it was in two contigs (Table 4). As pRBH2–3 does not include an identifiable *rep* gene it cannot be typed using the recently described typing tool.

3.7. pRBH2–4 is related to pABTJ2 and encodes an R3-T3 rep

The complete structure of the 80,234 bp plasmid pRBH2–4 which also carries no antibiotic resistance genes is shown in Fig. 3A. The *rep* gene is identical to the one in the larger (110,967 bp) cryptic plasmid pABTJ2 (CP004359; Huang et al., 2014). The Rep protein of pABTJ2 is the representative of the R3-T3 type, a plasmid type commonly seen in *A. baumannii* isolates (Lam et al., 2023). In pRBH2–4, 8 imperfect 18 bp iterons separated by 4–191 bp were found located upstream of the *rep* gene (Table 5). Previously, four imperfectly conserved 22 bp direct repeats were reported in pABTJ2 but their location was not indicated (Huang et al., 2014). The Rep proteins in R3-T2 (pRBH2–2) and R3-T3 plasmids are only 41.5% identical over approximately 70% of the protein and they are clearly compatible as the presence of an R3-T2 and an R3-T3 plasmid together is not uncommon (e.g. in J9 (Hamidian et al., 2021) and WM99c (Nigro et al., 2018)).

The first half of pRBH2–4 is closely related to part of pABTJ2 (CP004359), with large regions of >97% DNA identity though there are some small regions of difference (Fig. 3B). In the second half of the plasmid only small segments are shared with pABTJ2 and there are large

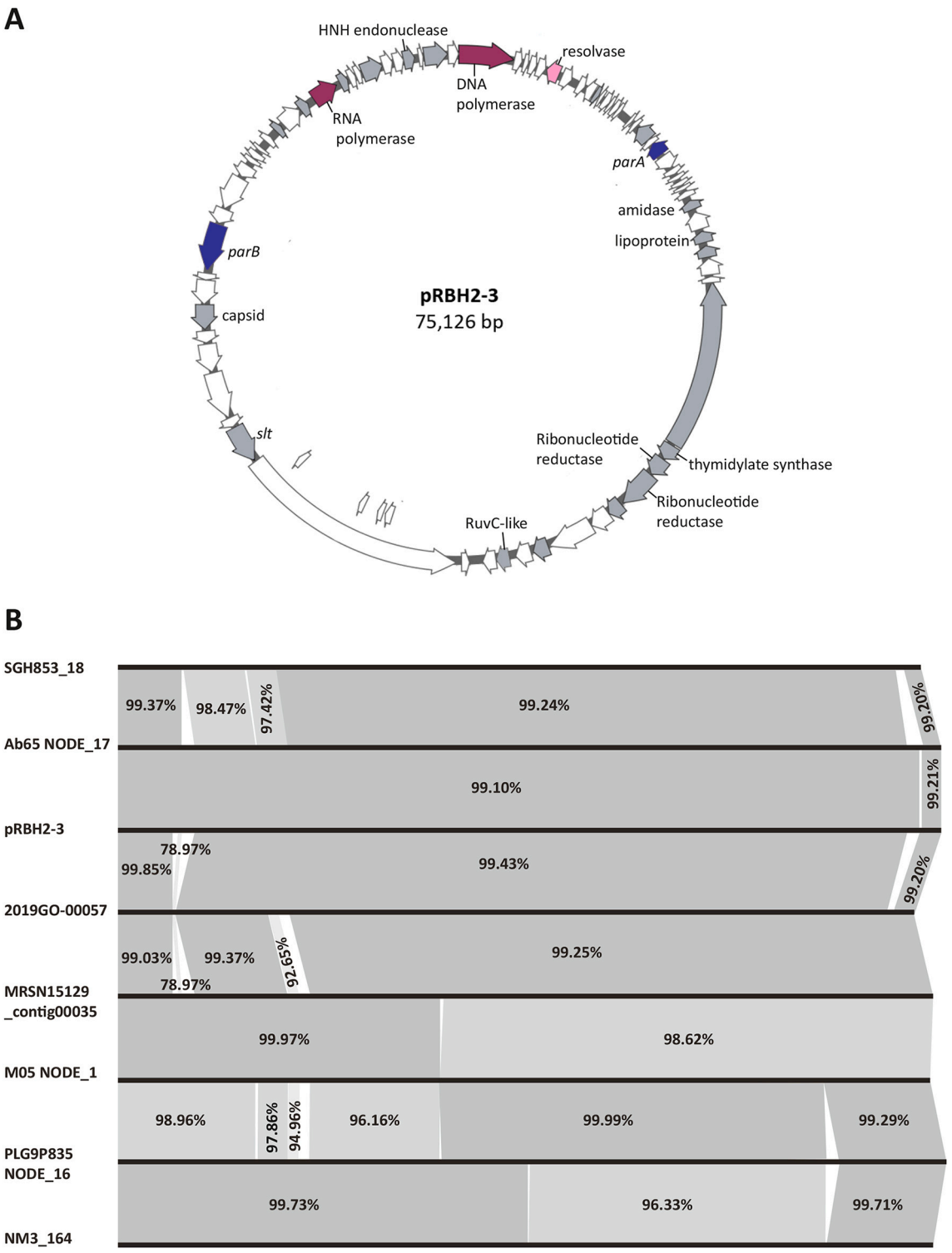


Fig. 2. Structure of pRBH2–3 and comparison to available sequences in WGS. (A) Map of plasmid pRBH2–3. Genes are marked by arrows indicating their extent and orientation. Genes involved in DNA metabolism are purple, the putative partitioning genes are blue. The gene encoding a putative serine recombinase is pink, genes which encode proteins with a putative function are grey and all other reading frames are white. (B) Comparison of related plasmid sequences available in WGS. Plasmid sequences are indicated by black lines, with the name of the plasmid adjacent. Regions of high DNA identity are indicated by grey shading with the percent identity also shown. The figure is drawn to scale from GenBank accession numbers listed in Table 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

regions of difference between the two plasmids (Fig. 3B). In pRBH2–3, a 9.3 kb region has replaced 1025 bp of the pABTJ2 backbone (Fig. 3C). This replaced region is in precisely the same location as the *sul2* resistance region (4.6 kb) in pJ9–3 (CP041590; Hamidian et al., 2021), another pABTJ2 related plasmid. The 9.3 kb region in pRBH2–4 includes several IS, a hybrid of IS*1006* and IS*1008* at the left-hand boundary, a copy of IS*1008* and as well as partial copies of IS*1006* and IS*17* (Fig. 3C). Detailed examination of the right end of this region failed to provide any

Table 4
Relatives of pRBH2–3 with sequences available in WGS.

Name	Source species	ST[IP]	Contig length	Overlap	Plasmid size	Country	Year of isolation	Isolation source	Accession
pRBH2–3	*A. baumannii*	111	75,126	–	75,126	Australia	1999	–	CP110465
SGH0823_18 (S30)	*A. baumannii*	2	73,329	127 bp	73,202[a]	Singapore	2008	Endotracheal aspiration	PYEJ01000018
MRSN4943_contig00057	*A. baumannii*	2	73,202	–	73,202[a]	USA	2011	Respiratory	VHEM01000057
MRSN11660 SAMN04485904-rid12666013	*A. baumannii*	2	73,202	–	73,202[a]	USA	2009	Blood	AAYNPJ020000015
MRSN15129_contig00035	*A. baumannii*	1126	74,454	–	74,454	Peru	2012	Respiratory	VHGR01000035
PLG9P835 NODE_16	*A. baumannii*	1179	75,628	127 bp	75,501	Poland	2016	White Stork Choana	NIWR01000016
NIPH 1362 genomic scaffold acLZD-supercontig1.7	*A. baumannii*	47	66,201 8245	–	-[b]	–	–	–	KB849246 KB849245
NM3_164	*A. baumannii*	25	74,490	127 bp	74,363	UAE	2008	Sputum	JZBV01000040
M05 NODE_1	*A. baumannii*	2	74,266	127 bp	74,139	Egypt	2020	Blood culture	JAESHO010000001
2019GO-00057 SAMN14669247-rid9743133.denovo.017	*A. baumannii*	46	72,599	–	72,599[c]	USA	2019	Sputum	AAYMGQ010000017
2019GO-00115 SAMN14669304-rid9743673.denovo.017	*A. baumannii*	46	72,599	–	72,599[c]	USA	2019	Sputum	AAYMFV010000017
Ab65 NODE_17	*A. baumannii*	221	75,311	99 bp	75,212	China	2020	Respiratory	JANWCH010000017

[a] MRSN4943_contig00057, MRSN11660 SAMN04485904-rid12666013 and SGH0823_18 (S30) differ from one another by 1–2 SNPs.
[b] Plasmid size not determined as match to pRBH2–3 is across 2 contigs.
[c] 2019GO-00057 SAMN14669247-rid9743133.denovo.017 and 2019GO-00115 SAMN14669304-rid9743673.denovo.017 share 100% DNA identity.

insight into how replacement of the pABTJ2 backbone segment initiated by the IS*1006/1008* hybrid may have been completed at this end. In the additional region in pRBH2–4 there is an *eptA* gene which encodes a putative ethanolamine phosphotransferase that shares >65% aa identity with the two EptA variants encoded by genes in *Acinetobacter* that have been reported to play a role in colistin resistance (Trebosc et al., 2019). However, RBH2 is colistin susceptible (MIC 0.125 mg/L). Elsewhere in pRBH2–4 there are also copies of ISAba34, ISAba11, IS*26* and a partial copy of ISAha2 (Table 3).

Comparison of the inserted regions in pRBH2–4 and pJ9–3 (Fig. 3C) revealed that the segment between the hybrid IS*1006*/IS*1008* and ISAba1 in pJ9–3 represents an internal replacement as the short segment between ISAba1 and IS*17*Δ in pJ9–3 is present in pRBH2–4. The IS*1006*/IS*1008* hybrid in pRBH2–4, a mosaic of the two IS, is different to the IS*1006*/IS*1008* hybrid previously reported in pJ9–3 (Hamidian et al., 2021). In pJ9–3, the IS1006/1008 hybrid consists of bases 1–176 of IS*1006* followed by bases 171–820 of IS*1008*, whereas the hybrid IS in pRBH2–4 consists of bases 1–85 of IS*1006*, 73–230 of IS*1008*, bases 219–571 of IS*1006* and bases 542–820 of IS*1008*. However, the first 279 bp of the ISs at the shared boundary in both pJ9–3 and pRBH2–4 match IS*1008* and this provides a possible location for recombination at the left end.

3.8. pRBH2-5 – an RP-T1 (RepAci6) type plasmid with a type 3 backbone

pRBH2–5 is a 124,107 bp RepAci6 type plasmid, recently classified as RP-T1 type, that is closely related to pS21–2 (MG954377; Blackwell and Hall, 2019). pS21–2 has a copy of ISAba12-v1 in an intergenic region between reading frames located downstream of the *trwC* relaxase gene (Fig. 4A), and a 9 bp TSD surrounds the ISAba12-v1 sequence. However, in pRBH2–5 a deletion mediated by ISAba12-v1 has led to the loss of 1598 bp of the backbone relative to pS21–2. In pRBH2–5 two additional IS, namely ISAba1 and ISAba125, have interrupted the relaxase gene *trwC*, and are flanked by a 10 bp TSD (TSD generated by insertion of ISAba1 is usually 9 bp) and 3 bp TSD, respectively. These insertions would have rendered the plasmid non-conjugative. Removal of transposons, IS and one copy of any TSD generated a backbone of 68,353 bp for pRBH2–5, whereas the pS21–2 backbone is 69,951 bp. Apart from the ISAba12-v1 mediated deletion, the only other difference in the backbone is a single SNP at base 1612 of the *traC* gene (A in pS21–2 replaced by G in pRBH2–5).

In pRBH2–5 and pS21–2, AbaR4, carrying *oxa23* in Tn*2006*, interrupts an open reading frame which encodes a 372 aa hypothetical protein (Fig. 4A) and is flanked by a 5 bp TSD. In addition, a putative copper resistance transposon, flanked by a 5 bp TSD, interrupts the *umuC* gene in both (Fig. 4A). However, in pRBH2–5, the copper transposon (Tn*6177*-v1) differs from Tn*6177* found in pS21–2 in the presence of two diverged segments that appear to be derived from the Tn*6175* in the chromosome (Fig. 5B). For further comparison of the various copper resistance transposons see Section 3.10.

The backbone of pS21–2 (and pRBH2–5) shares a large amount of similarity with the backbones of the previously described RepAci6 plasmids pAbG7–2 (KF669606; Hamidian et al., 2014b) and pA85–3 (CP021787; Hamidian et al., 2014a), respectively, but there are some significant differences between the three plasmids suggesting the existence of three backbone types (Fig. 4B). pAbG7–2 and pA85–3 are related RepAci6 plasmids that consist of 3 backbone segments separated by three copies of a repeat region, two complete (R2, 422 bp; R3, 423 bp) and one partial (R1, 225 bp) (Hamidian et al., 2014a) but there are a number of differences between the two plasmids which can be distinguished based on SNPs throughout the backbone as well as short insertions and differences in the sequence around the third repeat (Fig. 4C). Hence, pAbG7–2 and pA85–3 are representative of two backbone types which we designate Type 1 and Type 2, respectively.

pRBH2–5 and pS21–2 represent a third type. They have an additional partial copy of the repeat region (R4; 179 bp) and an additional 2.2 kb of sequence that includes two open reading frames (Fig. 4B). In pS21–2, the region from the start of the plasmid (opened at the start of the *rep* gene) to the first repeat (R1) and from R3 to the end of the plasmid share 99.24% and 99.28% DNA identity respectively, with pAb-G7–2 (Fig. 4B). There is greater variation between R1 and R3 than in the rest of the backbone with an increase in the number of SNPs, as well as several short insertions or deletions (Fig. 4B).

3.9. pRBH2–6 is a close relative of pAB3

pRBH2–6 is the largest plasmid in RBH2 at 141,033 bp and has a copy number approximately equal to that of the chromosome (Table 1). A complete map of this plasmid is shown in Fig. 6A. pRBH2–6 is closely related to the large conjugative plasmid pAB3 found in ATCC17978 (CP012005; Weber et al., 2015) which has no known replication initiation gene. Consequently this plasmid group is not detected by the *Acinetobacter* plasmid typing tool. The resistance genes in pRBH2–6 are

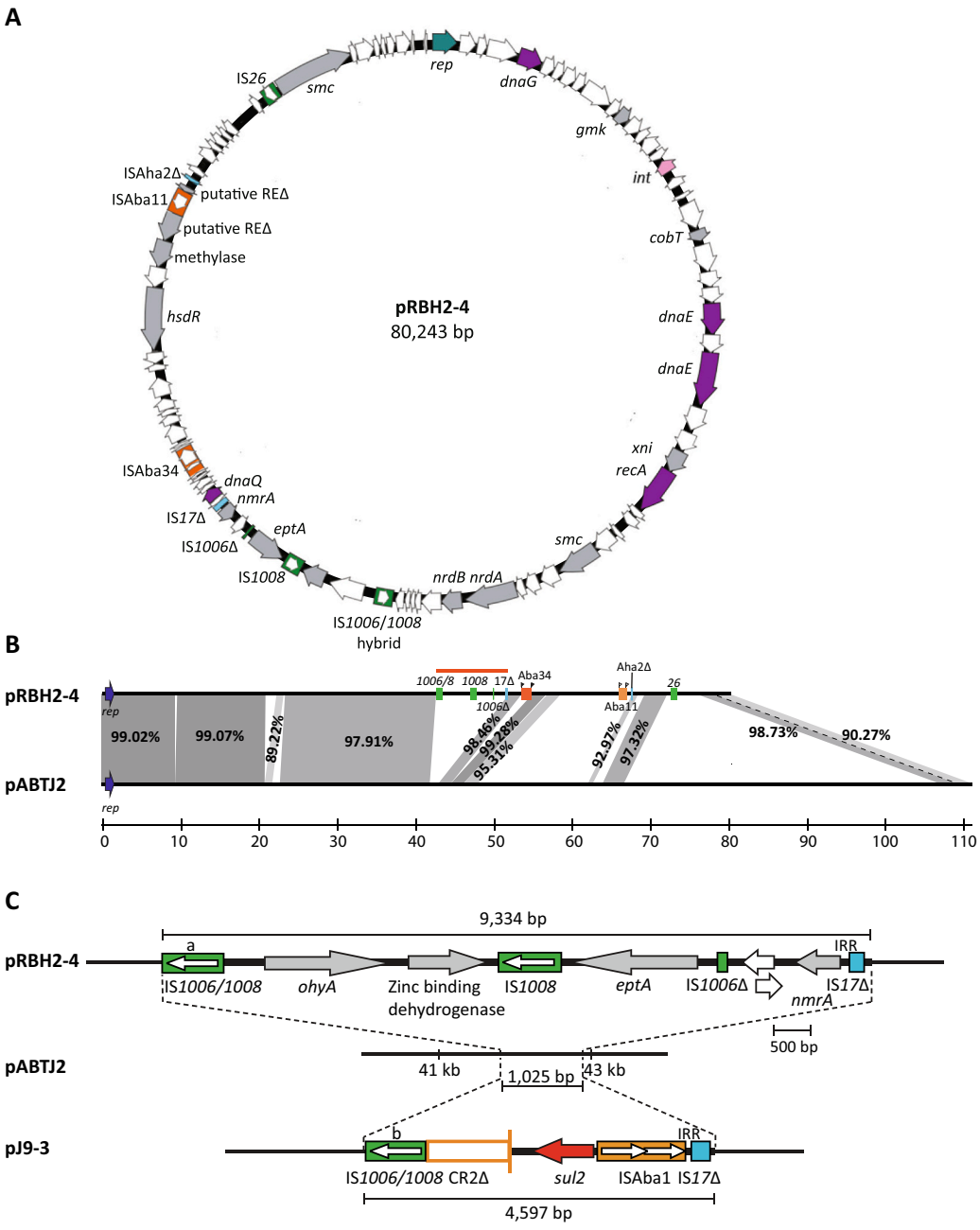


Fig. 3. Structure of pRBH2–4. (A) Map of plasmid pRBH2–4. Genes are shown as arrows which indicate their extent and orientation. The replication initiation gene is green, *dnaG*, *dnaE* and *recA* genes are purple and the *int* gene is pink, other genes are grey and reading frames that encode hypothetical proteins are white. IS are shown as boxes with an arrow indicating the extent/orientation of the transposase gene, IS*26*, IS*1006/08* and IS*1008* are green, IS*17*Δ is blue and ISAba11 and ISAba34 are orange. (B) Comparison of pRBH2–4 and pABTJ2. Black lines represent the plasmid sequences with the name adjacent. The extent and orientation of the *repA* gene is indicated by a blue arrow. The region inserted in pRBH2–4 is indicated by an orange bar above the pRBH2–4 sequence. Regions of significant DNA identity are indicated by grey shading and the percent identity is shown. IS are indicated by coloured boxes as in (A). When present, target site duplications are indicated by black flags. A scale bar is shown below. (C) Additional region in pJ9–3. A thin black line represents the backbone sequences and the backbone region replaced in pJ9–3 and pRBH2–4 are delineated by dashed lines. Genes and IS are indicated as in (A) with the *sul2* resistance gene indicated by a red arrow. The partial CR2 in pJ9–3 is represented by an open orange box with the *ori* end indicated by a vertical orange bar. A thick black line represents the additional region in pRBH2–4 and pJ9–3. Horizontal bars indicate the extend of segments with the size of each region indicated above. The numbers below the pABTJ2 segment represent location in the pABTJ2 GenBank Entry (CP004359). The figures are drawn to scale from GenBank Accession numbers CP110465 (pRBH2–5), CP004359 (pABTJ2) and CP041590 (pJ9–3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Iteron sequences in pRBH2–4.

Iteron	Sequence[a]	Bases to next iteron
1	ACGGTACAAAAATTCCGT	96
2	AAGGTACAAAAACTCCGT	47
3	ACGGTACAAATATTCCTT	4
4	ACGGTACAAAAATTCTGT	21
5	AAGGTACAAAAACTCCGT	191
6	ACGGTACAAAAACTCCGA	46
7	ACGGTACAAAAATTCCGA	13
8	ACGGTACAAAAACTCCGG	–
Consensus	AcGGTACAAAaAYTCcgt	

[a] Bold indicates bases which are not shared by all 8 iterons; iteron region from bases 79,542–80,102 in CP110466. There are 141 bp between the end of the iterons and start of the *rep* gene.

found in 2 transposons separated by a 6.2 kb segment of plasmid backbone and pRBH2–6 differs from pAB3 mainly in these regions.

The first transposon in pAB3 is Tn*6021* (Hamidian and Hall, 2017). However, in pRBH2–6 it has acquired Tn*2006* (*oxa23*), to become AbaR4 (consists of Tn*6022* interrupted by Tn*2006*), with a copy of ISAba11 inserted in the *tniC* gene at the same location as observed in Tn*6021* (Fig. 6C).

The second transposon is related to the resistance transposon Tn*6172* (Hamidian and Hall, 2017) which carries the *sul2*, *strA* and *strB* resistance genes. The *tet*(B) determinant has also come into this transposon likely by homologous recombination between two CR2 segments (Fig. 6C). pAB3 has a related transposon, Tn*6174* at the same location (Fig. 6C). In both pRBH2–6 and pAB3, the two transposons are separated by a 6.2 kb segment which is identical to the central segment in the AbGRI1 resistance island (Hamidian and Hall, 2017). The structure in pRBH2–6 is close to the previously proposed progenitor of the AbGRI1

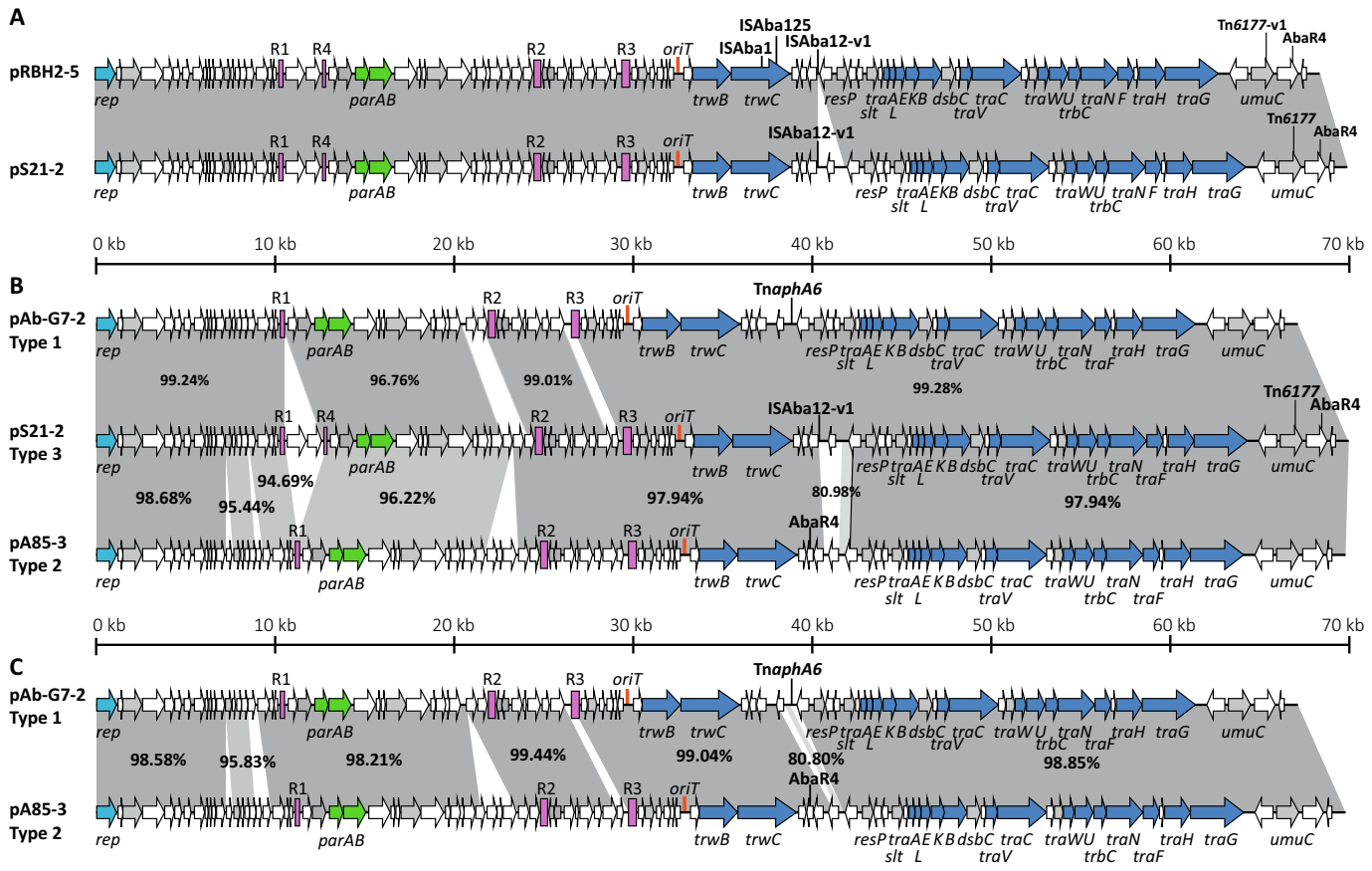


Fig. 4. Comparison of the pRBH2–5 with other RepAci6 plasmids. Comparison of (a) pRBH2–5 and pS21–2, (B) pS21–2, pAb-G7–2 (Type 1) and pA85–3 (Type 2), and (C) pAb-G7–2 and pA85–3. Arrows indicate the extent and orientation of the genes and open reading frames. The *rep* gene is light blue, genes involved in conjugal transfer are dark blue; partitioning genes are green and other genes with known functions are grey, all orfs that encode proteins with no known function are white. Green boxes represent the repeat sequences in the RepAci6 backbone with their repeat number above. Vertical lines show the location of insertions (transposons or IS) with their name above. *oriT* is indicated by a red bar. Regions of significant DNA identity are indicated by grey shading with the percent identity of each region shown. Figure drawn to scale from GenBank Accession numbers CP110467 (pRBH2–5), MG954377 (pS21–2), KF669606 (pAb-G7–2) and KJ493819 (pA85–3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

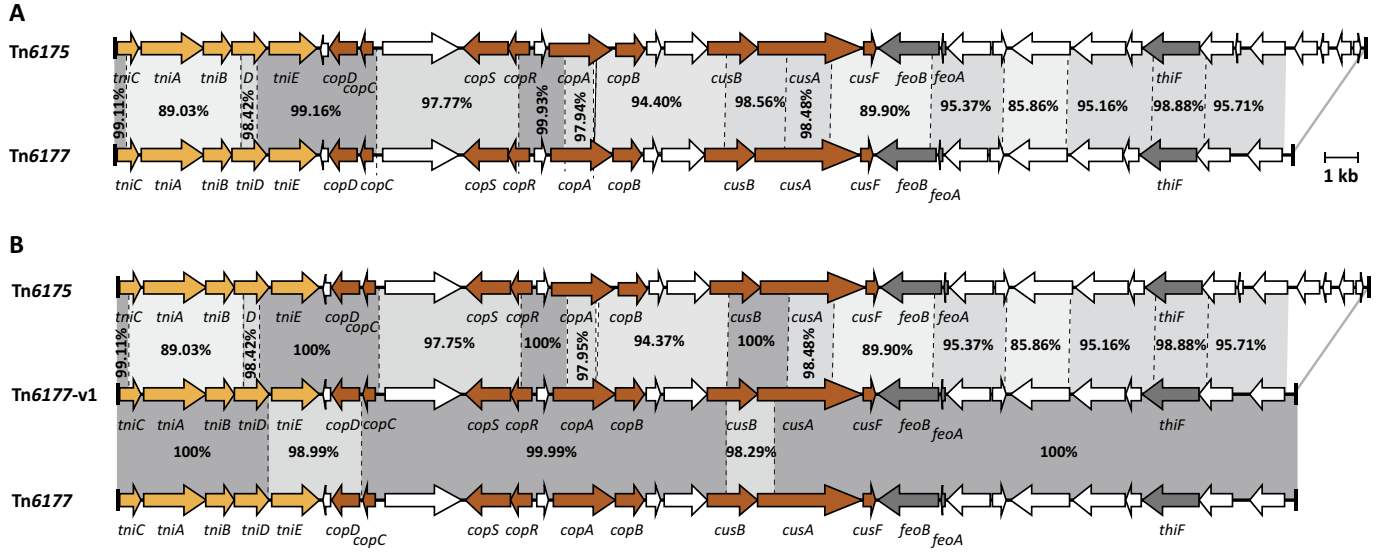
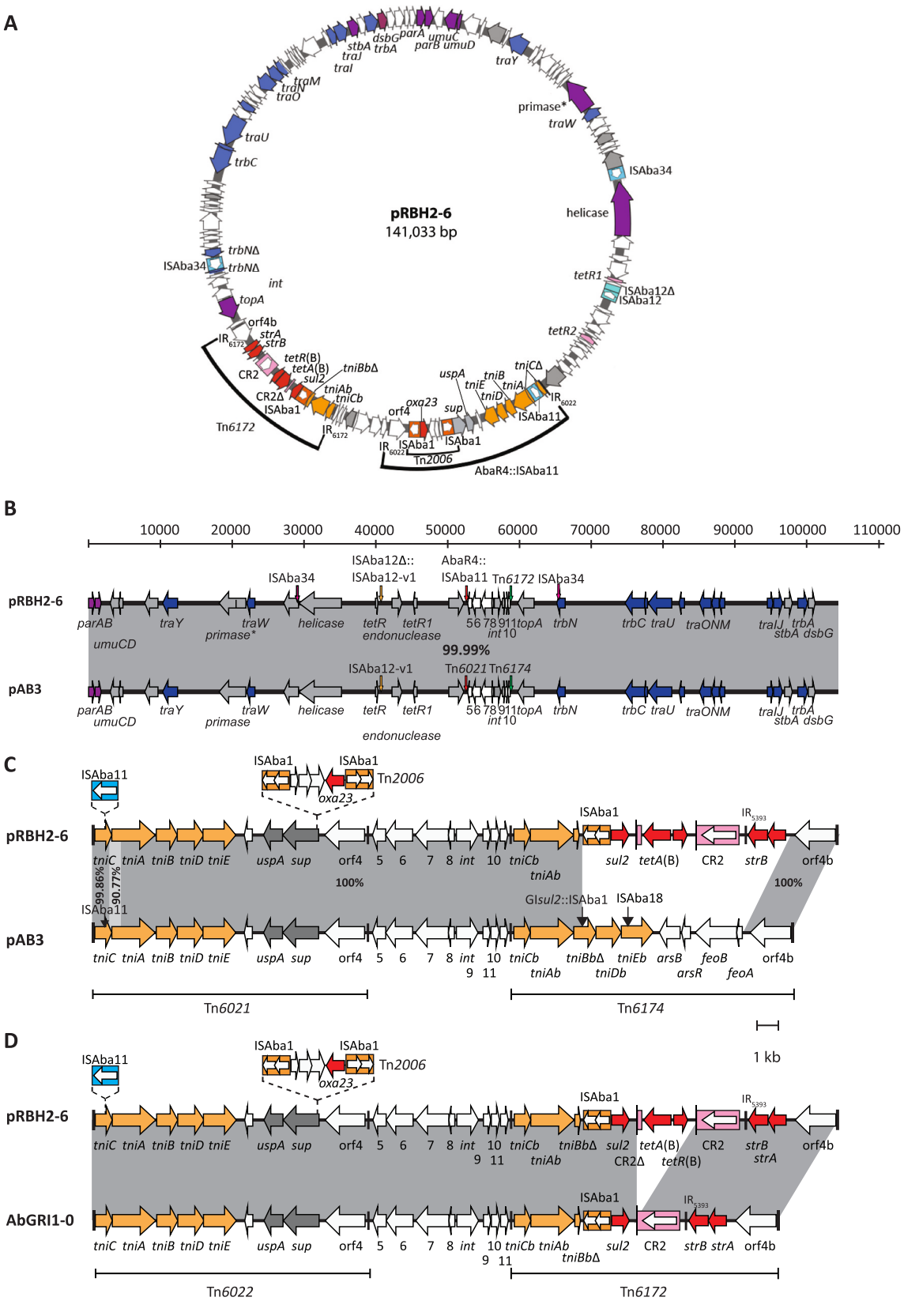


Fig. 5. Structure and comparison of copper resistance transposons in RBH2. Comparison of (A) Tn*6175* and Tn*6177* and (B) The copper resistance transposon in pRBH2–5 with Tn*6175* and Tn*6177*. Transposons are indicated by a black line with the vertical bar at either end indicating the inverted repeats. Arrows indicate the extent and orientation of genes and orfs. Genes involved in transposition are orange, genes likely to be involved in copper resistance are brown, other named genes are grey and all remaining orfs are white. Differing shades of grey are used to show regions of significant DNA identity and the percent identity is also shown. Dashed lines delineate regions of differing identity. Transposon regions are drawn to scale from GenBank accession numbers CP110462 (Tn*6175*), CP110467 (Tn*6177*–v1) and MG954377 (Tn*6177*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



(caption on next page)

Fig. 6. Structure of pRBH2–6 and comparison to pAB3. (A) Map of plasmid pRBH2–6. The extent and orientation of genes and open reading frames are indicated by arrows. Genes involved in DNA metabolism are purple, genes involved in conjugal transfer are blue, genes that encode TetR family transcriptional regulators are pink, other named genes are grey and genes that encode hypothetical proteins are white. Genes involved in transposition are orange and resistance genes are red. IS are indicated as coloured boxes with an internal arrow indicating the orientation of the transposase gene. CR2 is pink with the *ori* end indicated by a vertical black bar. IRs are shown as vertical bars and the extent of Tn*6172*, AbaR4::ISAba11 and Tn*2006* are indicated. Figure is drawn to scale from GenBank accession number CP110468. (B) Comparison of the pRBH2–6 backbone with the pAB3 backbone. Genes and orfs are indicated as in (A). The location of transposons and insertion sequences are indicated by coloured arrows with the name above. Regions of DNA homology indicated by grey shading with the percent identity shown. Comparison of pRBH2–6 resistance region with (C) the pAB3 resistance region and (D) AbGRI1–0. Features are indicated as in (A). The extent of each transposon is shown below and vertical lines at ends indicate IRs. Regions of significant identity are indicated as in (B). A scale bar is shown. Figure is drawn to scale from GenBank accession numbers CP110468 (pRBH2–5) and CP012005 (pAB3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

resistance island (Hamidian and Hall, 2017) though there have been some additions to the two transposons, with ISAba11 and Tn*2006* inserted into Tn*6022* and *tet*(B) in Tn*6172* (Fig. 6D).

pRBH2–6 and pAB3 both have a copy of ISAba12-v1 downstream of one of the *tetR* regulator genes (Fig. 6B). However, in pRBH2–6 there is an additional partial copy of this IS adjacent to the complete copy. In addition, pRBH2–6 has two copies of ISAba34, both flanked by a 5 bp TSD. One is in an intergenic region downstream of a gene encoding a helicase and the second copy has interrupted the *trbN* gene (Fig. 6B), which is thought to be involved in conjugative transfer. pAB3 has been shown to be conjugative (Weber et al., 2015) but pRBH2–6 is not likely to be conjugative as *trbN* is a conjugative transfer gene.

Removal of transposons and IS in pRBH2–6 leaves a backbone of 104,478 bp. The backbone of pRBH2–6 is very closely related to the backbone of pAB3 (104,476 bp) with 99.99% identity across the entire backbone (Fig. 6B). There are only 5 differences between the two backbones, three single base changes and two single base insertions/deletions.

3.10. Tn*6175* and Tn*6177-v1*

We have previously reported three related putative copper resistance transposons, Tn*6175*, Tn*6176* and Tn*6177*. Two of these transposons, Tn*6175* (37,814 bp) and Tn*6177* (35,611 bp), are closely related (Fig. 5A) and share 95% coverage and 96.3% DNA identity (Hamidian et al., 2021). Tn*6176* (35,611 bp), found in pC13–2 (KU549175) is more diverged with 87% coverage and 89.4% identity compared to Tn*6175* (Hamidian et al., 2021). The putative copper resistance transposon in pRBH2–5 is a variant form of Tn*6177* which was found inserted at the same location in pS21–2. The majority of this transposon shares >99.99% DNA identity with Tn*6177* (Fig. 5B). However, there are two regions where the DNA identity is lower (Fig. 5B).The first is a 2662 bp (2660 bp in Tn*6177*) region that includes the end of *tniD*, *tniE*, an *orf* and *copD* which is 98.99% identical to the corresponding region in Tn*6177* (Fig. 5B). The second segment (1466 bp) includes part of *cusB* and *cusA* and shares 98.29% DNA identity with Tn*6177*. However, these two regions are 100% identical to the corresponding regions in Tn*6175* (Fig. 5B). Hence, in pRBH2–5 these segments of the transposon are likely to have been replaced by homologous recombination.

3.11. Related ST111 isolates

The genome sequences of three additional ST111 *A. baumannii* isolates are available in WGS (last searched January 2023), two from Russia and one from Germany. These isolates were recovered between 2002 and 2014 (Table 6). All three include KL19 and OCL1 but differ from RBH2 in the Oxford MLST typing scheme; 28 and AB1347 are ST1167 which is a single locus variant (SLV) of ST1134 found in RBH2, while MRSN3874 is a SLV of ST1167. Searches for plasmids in these genomes revealed all three had an R3-T2 (RepAci2) type plasmid which is not the same as pRBH2–2. A RepAci6 plasmid (RP-T1) was identified in two strains but is more closely related to pAB-G7–2 (Type 1) than pRBH2–5. None of the three strains have a plasmid related to pRBH2–6, pRBH2–3, pRBH2–4 or pRBH2–1 (pRAY*-v1). A number of resistance genes were identified in each of the three strains (Table 6) but their location (on the chromosome or plasmids) could not be determined from the available contigs.

To confirm that the ST111 group did not belong to either GC1 or GC2, the average nucleotide identity between RBH2, A1 (CP010781; Holt et al., 2015) representing GC1, and WM99c (CP031743; Nigro et al., 2018) representing GC2 was calculated. The average nucleotide identity between A1 and WM99c was 97.79%. The average nucleotide identity between RBH2 and A1 or WM99c was 97.94% and 97.87%, respectively, confirming that RBH2 is not part of GC1 or GC2.

4. Discussion

RBH2 has a very large number of IS in its chromosome and two of them appear to be contributing to the resistance profile via activation of an adjacent gene. The role of ISAba1 upstream of the *ampC* gene is well documented (Hamidian and Hall, 2013; Hamidian and Hall, 2014), but the ISAba9 upstream of the *adeIJK* efflux operon increasing resistance to ciprofloxacin has not been noticed previously in clinical isolates. However, various other IS have been seen upstream of *adeIJK* after selection for ciprofloxacin resistance in an experimental study (Zlamal et al., 2021). Whether any other IS copies affect genes involved in virulence was not investigated.

Three of the transposons in the chromosome, AbaR4 and the two copies of Tn*6175*, carry a *tniC-tniA-tniB-tniD* transposition gene module and preferentially target the *comM* gene (Hamidian and Hall, 2011). In RBH2, the preferred site is occupied by Tn*6175*, and the other two transposons are in secondary sites. The Tn*7* variant carrying an additional Tn*2006* is in its preferred site downstream of *glmS*. A further copy of AbaR4 was found in pRBH2–5 together with another *tni*-dependent transposon related to Tn*6177*. An interesting feature of the Tn*6177*-v1 element is that the two patches of difference between it and Tn*6177*,

Table 6
Genomes of ST111^{IP}:KL19:OCL1 *A. baumannii* isolates.

Isolate[a]	Country	Year	Resistance genes	Plasmid Reps[b]	GenBank Accession number
28	Russia	2002	*strAB*, *aadB*, *aphA1*, *floR*	R3-T2 (RepAci2)	MAFT01000000
MRSN3874	Germany	2010	*mrsE*, *mphE*, *dfrA1*, *sul1*, *aadA1*, *aadB*, *aphA6*, *oxa23*	R3-T2 (RepAci2), RP-T1 (RepAci6)	VHET01000000
AB1347	Russia	2014	*strAB*, *aphA6*, *aadB*, *aphA1*, *sul2*	R3-T2 (RepAci2), RP-T1 (RepAci6)	DADAKK010000000

[a] RBH2 (ST1134^{Ox}) is a single locus variant of ST1167 found in 28 and AB1347. MRSN3874 is also a single locus variant of ST1167 (1 SNP in the *gyrB* allele).
[b] Plasmids pRBH2–1 (pRAY*-v1), pRBH2–3 and pRBH2–6, which are not identified by the plasmid typing scheme, were not detected in any of the three strains using nucleotide BLAST to search for the backbone of each plasmid against the whole genome of each isolate.

which is found in the same location in pS21–2, were identical to the corresponding segment of Tn*6175*. Hence, it can be concluded that they are derived from a Tn*6175* in the RBH2 chromosome (see Fig. 5). The TniCABD system of transposition has not been explored experimentally. However, it likely occurs by a "cut-out paste-in" mechanism based on the presence of the *tniC* gene which encodes a distant homologue (Pfam PF08722) of the TnsA protein of Tn*7*.

The detailed analysis of *Acinetobacter* plasmids is in its infancy. However, most include a gene encoding an identifiable Rep protein and a typing scheme for these has been reported recently. Overall the Rep proteins belong to only three Pfam groups and using a cutoff of 95% nucleotide identity for the *rep* gene, 6 Rep_1, 69 Rep_3 and 5 RepPriCT_1 types were found (Lam et al., 2023). Here, we found two Rep_3 plasmids (pRBH2–2 and pRBH2–4), and a single RepPriCT_1 plasmid (pRBH2–5).

The backbone of pRBH2–5 (and pS21–2) were related to previously described RepAci6 plasmids but had a number of differences. Consequently, we have identified three distinct plasmid backbone types among the RP-T1 (RepAci6) plasmid group and these three types had been noted previously as part of the analysis of pS21–2 (Blackwell, 2017). These distinctions will assist in tracking these conjugative plasmids that often carry transposons that include antibiotic resistance genes. The plasmids that lack a known *rep* gene, such as pRAY* and the pAB3/pA297–3 type, found here as pRAY*-v1 (pRBH2–1) and pRBH2–6, have not yet been systematically classified. Here, we have added a further type to this group in the form of pRBH2–3, which has a small number of close relatives in the WGS database (Table 4). In the case of the larger plasmids of this type, the protein or proteins required for replication remain to be identified and this could assist to draw them into a comprehensive typing scheme.

From an epidemiology perspective, it is interesting that the closest relatives of two of the plasmids in RBH2 are plasmids found in S21 (SGH9601) a clinical GC2 isolate from Singapore. As neither pS21–1/pRBH2–2 nor pS21–2/pRBH2–5 are common, it is possible that the RBH2 isolate was imported from Singapore or the South-East Asia region. Indeed, the two plasmids may have transferred together as RepAci6 plasmids can mobilise pS21–1 albeit at a low frequency (Blackwell and Hall, 2019). However, no transconjugants were detected for pS21–2 despite the fact that all known transfer genes are intact (Blackwell and Hall, 2019). Likewise, pAB3 and pA297–3 have been shown to mobilise pRAY* (Hamidian and Hall, 2018a; Di Venanzio et al., 2019), and pRAY*-v1 and pRBH2–6 may have entered RBH2 together.

Clearly, the already extensive repertoire of plasmid types that can be found in *Acinetobacter* spp. isolates is far from exhausted as new types continue to be identified, both here and recently (Harmer et al., 2022), over 10 years after the first small group of known sequences were classified by Bertini and co-workers (Bertini et al., 2010). However, five of the plasmid types found in RBH2 (R3-T2 (RepAci2), R3-T3, RP-T1 (RepAci6), pRAY* and pAB3/pA297–3 types) are quite widespread in other sequence types including the dominant GC1 and GC2 clones The unique repertoire of plasmids found in *A. baumannii* and more broadly in other *Acinetobacter* species has received little attention with respect to how they replicate and how they spread into new hosts. Three types of large potentially conjugative plasmids, RepAci6 plasmids, the pAB3/pA297–3 type and the NDM type have been shown to transfer into new hosts (Hu et al., 2012; Hamidian et al., 2014b; Hamidian et al., 2014a; Weber et al., 2015; Hamidian et al., 2016). Plasmids in these groups also contribute to the antibiotic resistance gene burden. However, for two of these plasmid types a *rep* gene has not been identified and experimental work will be needed to achieve this.

Overall, RBH2 belonging to a rare sequence type includes a high load of acquired elements (IS and transposons) in the chromosome but has remained able to cause an infection. A recent survey found only seven genomes with six or more plasmids among the 423 available complete *A. baumannii* genomes (Prity et al., 2023). Hence, the six plasmids in RBH2 is also a notably high number. RBH2 also illustrates the capacity of IS and transposons together with plasmids uniquely found in *Acinetobacter* species to generate an extensive antibiotic resistance phenotype.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plasmid.2023.102707.

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